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The Journal of Laboratory and Clinical Medicine

Clinical and Experimental

Radioactive Phosphorus as a Therapeutic Agent. A Review of the Literature and Analysis of the Results of Treatment of 155 Patients With Various Blood Dyscrasias, Lymphomas, and Other Malignant Neoplastic Diseases. Edward H. Reinhard, M.D., Carl V. Moore, M.D., Olga S. Bierbaum, B.S., and Sherwood Moore, M.D., St. Louis, Mo. With an Appendix by Martin D. Kamen, Ph.D., St. Louis, Mo. ---- 107

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RADIOACTIVE PHOSPHORUS AS A THERAPEUTIC AGENT. A REVIEW OF THE LITERATURE AND ANALYSIS OF THE RESULTS OF TREATMENT OF 155 PATIENTS WITH VARIOUS BLOOD DYSCRASIAS, LYMPHOMAS, AND OTHER MALIGNANT NEOPLASTIC DISEASES

EDWARD H. REINHARD, M.D., CARL V. MOORE, M.D., OLGA S. BIERBAUM, B.S., AND SHERWOOD MOORE, M.D.
ST. LOUIS, MO.

WITH AN APPENDIX BY MARTIN D. KAMEN, PH.D., ST. LOUIS, MO.

THE ASSAY OF RADIOACTIVE PHOSPHORUS

RADIOACTIVE phosphorus has been used for the treatment of patients with various diseases since 1936. Lawrence and his associates[1] published in 1939 the first detailed report of therapeutic results obtained with radioactive phosphorus; the four patients discussed in this report all had leukemia. Since then, many observers[2-17] have recorded additional experience in the treatment of leukemia and a wide variety of other disorders, principally malignant neoplastic diseases. These reports describe the effectiveness of this new therapeutic agent in a total of approximately 406 patients. Unfortunately, however, specific data in the form of case reports, charts, or graphs are given for only 140 of the subjects. It is the purpose of this communication to (1) review this literature critically, (2) summarize the experimental work which provided the rationale for the use of radioactive phosphorus, (3) describe our own experience accumulated during the past four years and based on the treatment of 155 patients who had various hematologic dyscrasias or malignant neoplastic diseases, and (4) evaluate the effectiveness of this therapy and comapre it with older methods of treatment. We realize that four years of personal and nine years of over-all experience is insufficient for the final evaluation of radioactive phosphorus as a method of treating polycythemia vera and the chronic forms of leukemia. But even for these diseases it seems profitable to summarize existing information and draw tentative conclusions.

From the Edward Mallinckrodt Institute of Radiology and the Department of Internal Medicine, Washington University School of Medicine.

The authors wish to express their indebtedness to a large number of persons whose cooperation and help made these studies possible. Louis H. Hempelmann, M.D., initiated this survey but had to leave shortly thereafter for government service. Arthur Hughes, Ph.D., Harry Fulbright, Ph.D., Albert Schulke, and Harry Huth regularly supplied us with radioactive phosphorus often at considerable personal sacrifice of time and convenience. Reubenia Dubach, Ph.D., and Mary Lou Wheeler kindly made the measurements of radioactivity in tissues. Elizabeth Rice Sido was of invaluable aid in the hematologic studies. Finally, indebtedness is acknowledged to the many physicians who referred patients to us and made it possible for us to follow the patients at frequent intervals.

This investigation was aided in part by a grant from the Commonwealth Fund.

Received for publication, Dec. 20, 1945.

For the sake of clarity, the various hematologic dyscrasias for which radioactive phosphorus has been used therapeutically will be treated separately. Under each such heading, the pertinent literature will be reviewed and our own data given. Material will be presented in the following order:

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I. MATERIAL AND METHODS

The diagnoses established for the 155 patients included in our own study were: polycythemia vera, 30; myelogenous leukemia, 39; lymphatic leukemia, 45; monocytic leukemia, 10; Hodgkin's disease, 6; lymphosarcoma (small cell type), 5; reticulum cell sarcoma, 3; giant follicular lymphoblastoma, 1; multiple myeloma, 8; Ewing's sarcoma, 3; malignant melanoma, 1; anaplastic carcinoma (primary site undetermined), 1; mycosis fungoides, 2; and xanthomatosis, 1. The diagnosis in every case was confirmed by sternal bone marrow aspiration or by lymph node or tissue biopsy. Of these 155 patients, 82 have died and 73 are still living. Post-mortem examinations were performed on 53 of the 82 patients who have died.

The radioactive phosphorus was prepared in the cyclotron of the Mallinckrodt Institute of Radiology by bombardment of red phosphorus with 12 million volt deuterons. The phosphorus was then synthesized into its dibasic sodium salt, according to the procedure used by Kamen,[18] recorded in detail in a previous publication.[19] Sufficient water was added to make an isotonic solution (15 mg. Na_2HPO_4 per cubic centimeter). The radioactivity of the phosphate solution was determined by means of a Lauritsen electroscope calibrated with a uranium standard.* The initial activity of a freshly prepared solution usually varied between 0.20 and 0.40 millicuries per cubic centimeter. The solution of radioactive phosphorus was used until its strength had decayed to from 0.04 to 0.05 millicuries per cubic centimeter.

The plan of therapy for all the diseases except polycythemia vera closely followed the "fractional method" outlined by Low-Beer, Lawrence, and Stone.[12] This consists of small frequent intravenous injections of radioactive phosphorus. In only a very few instances was the material given by mouth. The size of each dose given parenterally varied between 0.1 and 2.5 millicuries (100 and 2,500 microcuries). Patients were usually treated two or three times a week at first. As the white blood cell count either approached normal in the patients with leukemia or became subnormal in those in whom the white blood count was not elevated at the beginning of therapy, the dosage was decreased and the time interval between treatments was lengthened. Patients with polycythemia vera were given larger quantities of radioactive phosphorus at much less frequent intervals.

Therapy was governed principally by the changes produced in the peripheral blood. In patients with leukemia and polycythemia vera, an attempt was made to restore the cytology of the blood as nearly to normal as possible and to maintain this effect. Considerable individualization of treatment was necessary because of the great variation in response shown by different persons. The remissions induced in most patients were sufficiently good to permit interruption of therapy for a period of months. In a few instances it seemed advisable to continue regular injections of the radioactive phosphorus at intervals of several

*It has been discovered that calibration of radioactivity is made differently at the Massachusetts Institute of Technology than at the University of California and at Washington University. Furthermore, the values obtained at the first of these institutions differ from those made at the other two. A more complete discussion of these differences is given in the appendix to this paper, written by Dr. Martin D. Kamen. Whenever dosage is given in terms of millicuries, this discrepancy must constantly be borne in mind.

weeks. More active therapy was reinstituted whenever the changes in the peripheral blood indicated that the remission had ended, even though no symptoms developed at these times.

In the cases of lymphosarcoma, reticulum cell sarcoma, Hodgkin's disease, multiple myeloma, and the other malignant neoplastic diseases, in which the white blood cell count was not elevated, regulation of therapy was more difficult. Administration of radioactive phosphorus was continued in these instances until changes in the peripheral blood gave warning of a depression of bone marrow activity. In some patients, serial bone marrow aspirations were done to aid in regulating therapy. Patients with subleukemic leukemia were given amounts that were comparable to those required by leukemic subjects with elevated leucocyte counts.

On each visit to the clinic, symptoms were recorded, the patient was examined, and the following laboratory data were obtained: total red blood cell and white blood cell counts, hemoglobin, reticulocyte level, platelet count, and a leucocytic differential. While fixed cover slip preparations were made for permanent records, the differentials were made routinely with the supravital technique. The blood-counting equipment had all been standardized by the U. S. Bureau of Standards. Hemoglobin determinations were made by the Evelyn oxyhemoglobin method[20] on an Evelyn photometer which had been standardized by determinations of the oxygen combining power. Reticulocyte and platelet counts were done by the Dameshek method.[21]

II. RATIONALE FOR THE THERAPEUTIC USE OF RADIOACTIVE PHOSPHORUS

A. *Definition of Radioactive Phosphorus and Description of Its Radiation Properties.*—The nature of radioactive phosphorus, the means by which it is prepared in the cyclotron, the nuclear transformation which occurs when it disintegrates, and the characteristics of the radiation emitted have been described simply and clearly by Aebersold.[22] In brief, to prepare radioactive phosphorus, ordinary stable red phosphorus is smeared on a copper target which is then placed directly in the path of a beam of high-speed deuterons (nuclei of heavy hydrogen) emitted by a cyclotron. As a result of this bombardment, a neutron enters the nucleus of a small fraction of the phosphorus atoms (Fig. 1). Stable phosphorus has an atomic mass of thirty-one—fifteen protons and sixteen neutrons, each of which has a mass of one. Since the mass of any atom is ordinarily written to the right and slightly above the atom's chemical symbol, stable phosphorus is referred to as P^{31}. When a neutron enters the phosphorus nucleus, the atomic mass is increased by one, and the resultant nucleus has a mass of 32. Radioactive phosphorus is, therefore, referred to as P^{32}; its nucleus is unstable and the atom is radioactive. This new nucleus has the same number of protons (positive charges) as the nucleus of P^{31}; thus, in the complete atom, there will be the same number of electrons (fifteen) revolving around the nucleus—one electron for each proton. The chemical identity of any atom is determined by the number of orbital electrons; P^{31} and P^{32}, therefore, are chemically identical. An atom of P^{32} eventually emits a beta-ray (an electron), whereupon one of the neutrons in the nucleus changes into a proton. The atomic mass of the nucleus

is not changed because protons and neutrons each have a mass of approximately one, but the positive charge on the nucleus is increased by one. This new nucleus is the nucleus of ordinary stable sulfur. The electron or beta-ray which is emitted when P^{32} is transformed into sulfur is the means by which radioactive phosphorus exerts its effect on tissues. These beta-rays have energies as high as 1.8 million electron volts; their average energy is approximately 0.6 million electron volts. The maximum range of penetration of these rays through the body tissues is approximately 0.7 centimeters. The half-life of P^{32} is 14.3 days, which means that within two weeks after administration any given dose of P^{32} will have lost half of its radioactivity by transformation into stable sulfur; within a month the activity will be one-fourth of the original value, etc. This rate of decay is short enough so that the radiation effects can be controlled, and yet it is sufficiently long so that a given tissue can be subjected to relatively prolonged, low-grade radiation. Thus, neoplastic or abnormally metabolizing cells can be subjected to a radiation effect throughout the whole of several mitotic cycles. If it is true, as has been suggested, that such cells are affected by radiation only during one phase of the mitotic cycle, then the prolonged, low-grade activity of P^{32} is an important theoretical advantage over x-ray therapy.

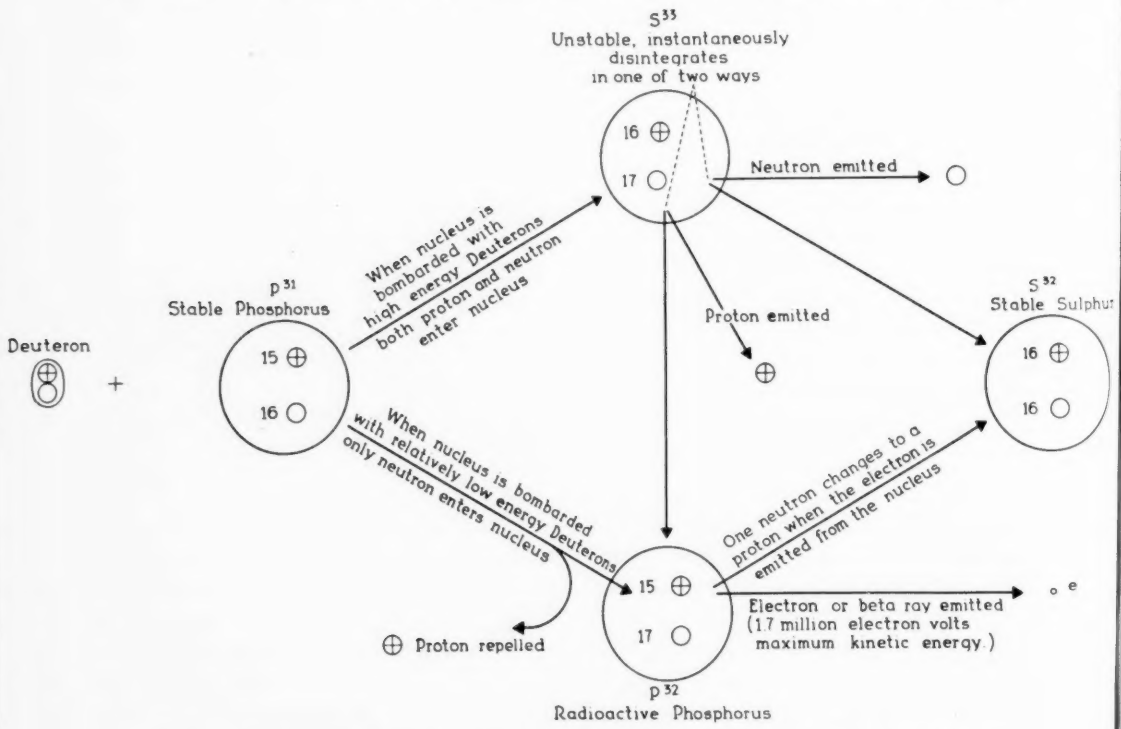

Fig. 1.—Radioactive phosphorus produced by deuteron bombardment of stable phosphorus (only nuclei are represented). Schematic presentation of nuclear changes involved in the production of radioactive phosphorus and its transformation into stable sulfur. Protons are marked with a plus because of their positive charge, while neutrons are left blank. The number of electrons which revolve about the nucleus (1) is equal to the number of protons in the nucleus and (2) determines the chemical identity of the atom. Since both stable and radioactive phosphorus have fifteen protons, they behave alike chemically.

When phosphorus is bombarded by deuterons in a cyclotron, only a small fraction of the atoms are made radioactive. The number depends on the number and energies of the striking deuterons. Actually, as the bombardment is carried out in the Washington University cyclotron, about one in every two million to five million atoms becomes P^{32}.

B. *Definition of Millicurie and Relation to Roentgens of X-ray.*—Since the dosage of radioactive phosphorus is always given in terms of millicuries, it is important that this unit of measurement be defined. A millcurie is that amount of any radioactive substance of which 37,000,000 atoms disintegrate per second[12] (see Dr. Kamen's discussion in the Appendix). Similarly, a microcurie is the amount which disintegrates at the rate of 37,000 atoms per second. This definition, it must be emphasized, does not take into account the type or energy of the radiation, both of which factors profoundly influence the physiologic effects of the radiation. For instance, radium emits alpha and gamma rays of high energy as well as beta-rays. One millicurie of radium, therefore, will produce different effects on tissue than will one millicurie of radioactive phosphorus. Consequently, one cannot assume that a millicurie of P^{32} will exert a radiation effect comparable to that of a millicurie of any other radioactive element or isotope.

An attempt has been made to translate these doses into terms with which clinicians and radiologists are more familiar. They have been related in particular to roentgens of x-rays.[5, 9, 11, 22] For example:

1. If 1.1 microcuries of P^{32} remain in a kilogram of tissue until it has completely disintegrated, 1 roentgen equivalent of radiation will be delivered to that tissue.

2. If 1 microcurie of P^{32} remains in a gram of tissue for twenty-four hours, 43 roentgen equivalents of radiation will be expended in that tissue.

3. If 1 millicurie of P^{32} is retained for twenty-four hours by an adult weighing 70 kilograms, the effect will be equivalent to 0.6 roentgen equivalents of whole body radiation.

These relationships, however, are certainly not exact since x-rays are usually administered from an external source to the surface of the body and penetrate to a variable depth, while the beta-rays of P^{32} are emitted from phosphorus actually in the tissue.

C. *Selective Uptake of Radioactive Phosphorus by Tissues and Cells.*—Fundamental studies by a number of investigators[23-29] have shown that P^{32} has a practical advantage over conventional methods of radiation therapy such as x-ray and radium in that when it is administered it is selectively withdrawn from the blood by certain tissues and cells. Fortunately, the tissues in which the greatest concentrations of radioactive phosphorus are deposited are usually those primarily involved in polycythemia vera, the leukemias, and the lymph node diseases. In general, this differential uptake of phosphorus by cells is dependent upon at least three factors: (1) the total amount of phosphorus in exchangeable form in the tissue, (2) the rate of turnover of phosphorus by the tissue, and (3) the rate at which new tissue is formed.

The extent to which the total exchangeable phosphorus in tissues influences the distribution of radioactive phosphorus is illustrated by bone. Bone has a

high inorganic phosphorus content and consequently takes up a large amount of the radioactive isotope. The effect of rate of turnover of phosphorus is illustrated by the marked difference in the accumulation of radioactive phosphorus in liver and brain. These two tissues have approximately the same phosphorus content, but liver takes up a large amount of phosphorus, whereas brain takes up relatively little due to the extremely slow rate of phosphorus metabolism by the brain. The effect of the rate at which cellular multiplication and new tissue formation occurs is illustrated by the fact that neoplastic tissue invariably takes up a great deal more phosphorus than does the same type of tissue in a normal state of growth.

Lawrence and his associates[26, 27] studied the distribution of radioactive phosphorus in the various tissues of normal and leukemic mice. A single dose of labeled phosphorus was administered to two groups of mice: one normal and one with lymphatic leukemia. The animals were sacrificed at various intervals, and the labeled (radioactive) phosphorus content of the tissues was determined. In Fig. 2 is shown the amount of radiophosphorus found in different organs expressed in per cent of the administered dose present per gram of wet tissue. There was only slightly more P^{32} in the bone and liver of the leukemic mice than in the corresponding organs of the normal animals. However, the lymph nodes and spleens of the leukemic animals took up from two to three times as much P^{32} as did these organs in the normal mice. This was interpreted as being due to the rapid laying down of new tissue as well as to the increased metabolic activity of the leukemic cells.

The distribution of radioactive phosphorus in the acid-soluble, phospholipid, and nucleoprotein fractions of tissues of normal and leukemic white mice has been studied by Tuttle and his associates.[27] A single dose of P^{32} was given intraperitoneally to each of a large group of normal and leukemic animals. The tissues were removed at varying intervals thereafter, the different organic phosphorus fractions were extracted, and the P^{32} content of each fraction was determined. In Fig. 3 is shown the distribution of the P^{32} in these different fractions. The nucleoprotein fraction of lymph nodes, spleen, and tumor tissue from the leukemic mice contained much more radiophosphorus than did the nucleoprotein fractions of corresponding organs in the normal animals. Thus, phosphorus (including radioactive phosphorus, if available) is taken up from the blood stream and used in the synthesis of nucleoproteins at a much more rapid rate by the leukemic cells than by cells in the corresponding normal tissues.

Marshak[28, 29] devised a technique for the separation of cell nuclei from cytoplasm and then employed this method to study the relative uptake of inorganic radioactive phosphorus in the nuclei and in the cytoplasm of normal and malignant cells (lymphoma, sarcoma 180, and carcinoma 256). He found that the nuclei of the malignant cells accumulated more of the labeled phosphorus than did those of normal cells. Furthermore, the relative proportion of the administered phosphorus in the nuclei as compared to that in the cytoplasm was much greater in the malignant than in the normal cells.

The distribution of radiophosphorus in the blood of patients has also been followed. In leukemia, Tuttle, Scott, and Lawrence[30] observed a rapid uptake

of the isotope by both red and white blood cells during the first twenty-four hours after administration. Within forty-eight hours, however, the concentration in erythrocytes began to decrease sharply, while that in the leucocytes continued to increase for an additional two to three days. At the end of nine days, the concentration per 100 cubic centimeters of white blood cells was between four and five times as great as in 100 cubic centimeters of erythrocytes. A determination made on the blood of one patient on the fifty-third day showed that this same ratio still held. It is of interest that phosphorus was present in the leucocytes during the first forty-eight hours largely in acid-soluble form. After that there was a gradual decrease in acid-soluble radioactive phosphorus together with a concomitant increase in the radioactivity of phospholipid and nucleoprotein fractions.[31] The concentration of P^{32} reached in both white and red cells was higher following intravenous than following oral administration. Relatively greater concentrations of P^{32} occurred in the nuclei than in the cytoplasm of myeloid leukemic cells, but no differences were noted in the nuclei and cytoplasm of lymphoid leukemic cells. When large amounts of stable phosphorus (P^{31}) accompanied the administration, the concentration of P^{32} which occurred in cells was decreased. From a practical point of view, therefore, the P^{32} given therapeutically should be accompanied by the smallest possible amount of P^{31}. Marrow was found to retain radiophosphorus in higher concentrations than blood. These observations apply largely to leukemic cells. Retention of radioactive phosphorus by red blood cells of normal individuals and of patients with polycythemia vera may be greater than that of leucocytes during the first twenty-four to forty-eight hours; shortly thereafter the ratio becomes inverted because of a decrease in the red cell fraction.[4] This decrease is apparently caused by a reduction in the radioactivity of the acid-soluble and nucleoprotein fractions; meanwhile, the P^{32} in the phospholipid fraction may actually increase slightly.

The distribution of radioactive phosphorus in the tissues of patients who had been treated with P^{32} has been studied by several groups of investigators. Leukemic patients who had received radiophosphorus shortly before death usually showed greatest concentrations of P^{32} in the bone marrow, lymph nodes, spleen, and liver.[9, 32-35] These findings were similar to those previously described for leukemic mice. Those patients, however, who had been given radioactive phosphorus many days before death frequently had the greatest concentrations of P^{32} in bone. It appears, therefore, that the isotope is first utilized by the more rapidly metabolizing tissues or those most frequently infiltrated with leukemic cells and that later it finds its way to bone. In lymphosarcoma, a selective uptake of P^{32} by lymph nodes has usually been observed,[8, 9, 13, 36] but in one patient studied by Erf and Lawrence[37] the mesenteric lymph nodes contained a smaller concentration than did many of the other tissues. In Hodgkin's disease, the findings have been variable and have apparently depended to some degree on the amount of fibrosis in lymph nodes. If the fibrotic change was advanced, small amounts of radioactivity were present in the nodes; if not, the lymph tissue contained concentrations comparable to those in rapidly metabolizing organs like liver and kidney.[37] Similar data have also been ac-

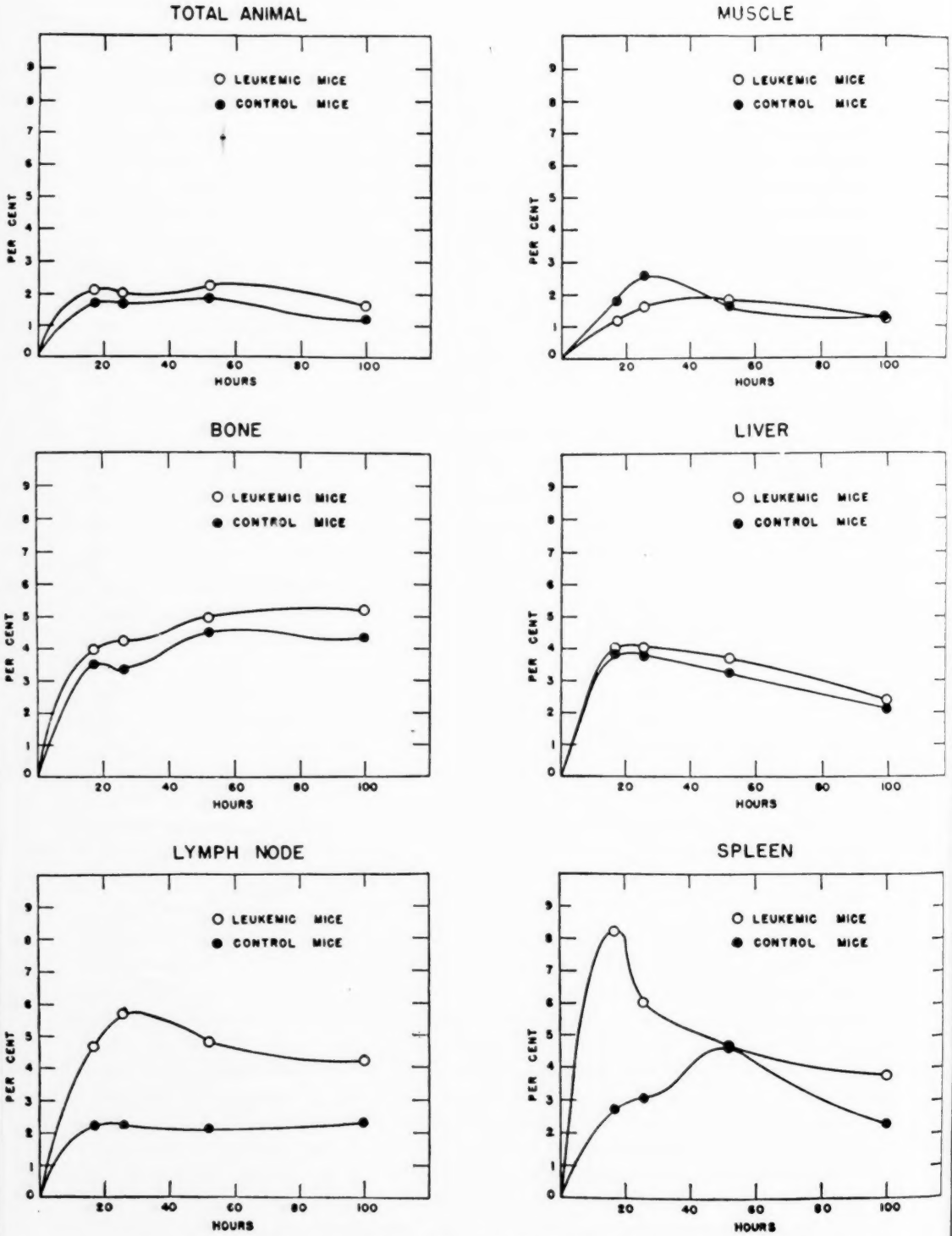

Fig. 2.—Distribution of radiophosphorus in the tissues of normal and leukemic mice. (Reprinted, with permission of Dr. John Lawrence, from J. Clin. Investigation 19: 267, 1940.)

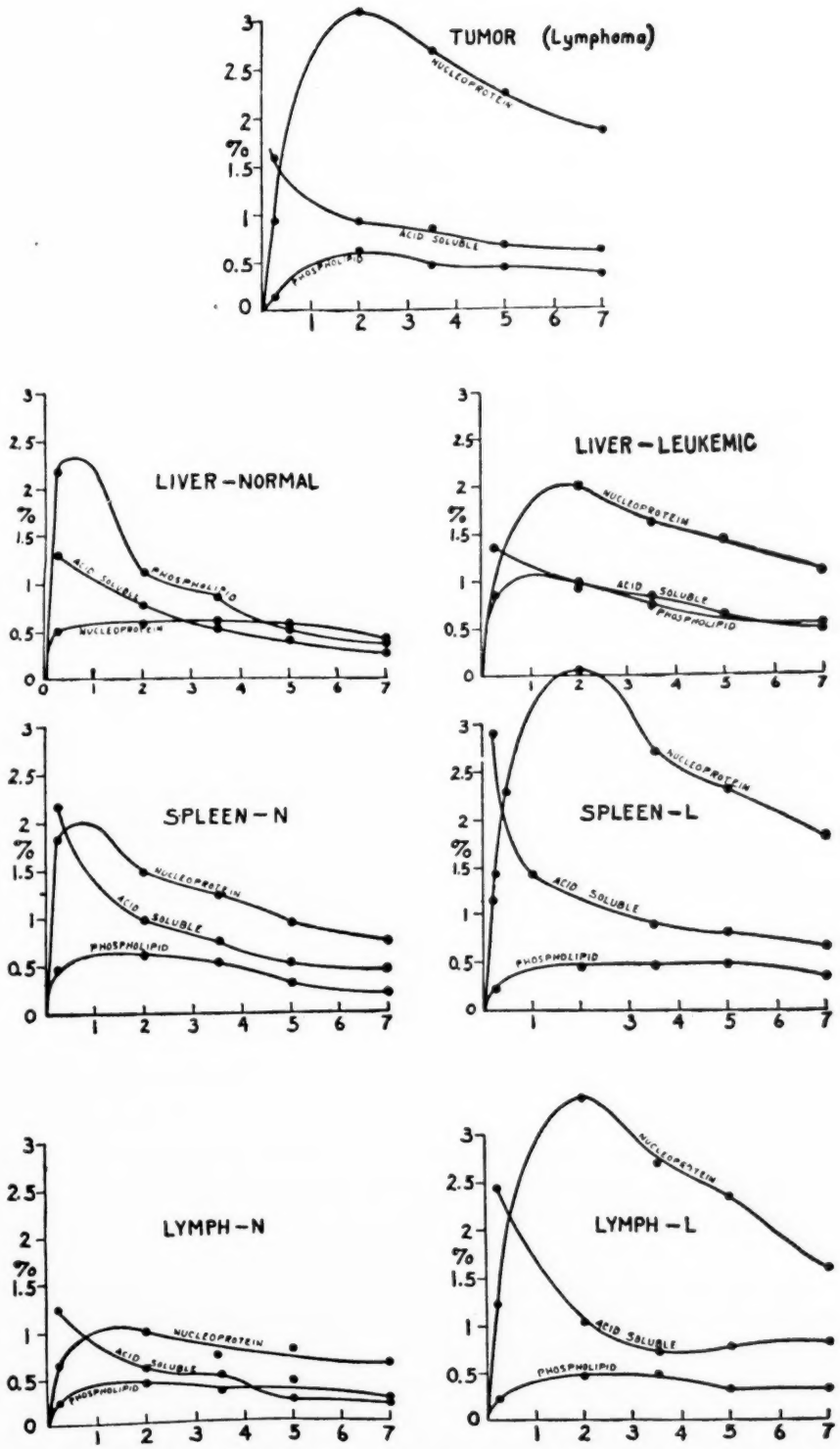

Fig. 3.—Phosphorus metabolism in neoplasms. The comparative uptake of labeled phosphorus in normal and leukemic mice. (Reprinted, with permission of authors, from J. Clin. Investigation 20: 57, 1941.)

TABLE 1. RADIOACTIVITY OF TISSUES OBTAINED AT AUTOPSY

PATIENT[a]	TOTAL DOSE (MC.)	TOTAL DURATION OF TREATMENT (WK.)	TIME SINCE LAST INJECTION OF P^{32} (DAYS)	BONE MARROW	BONE	LIVER	SPLEEN
Myelogenous leukemia							
5	19.703	74	6	.064 (1)	-	.043 (4)	.032 (5)
10	69.863	26	60	.0232 (2)	-	.0218 (3)	.0148 (4)
13	44.957	52	1	.294 (1)	.161	.218 (3)	.232 (2)
14	44.236	58	7	.144 (1)	.067	.132 (2)	.128 (4)
19	18.317	22	12	.0473 (1)	.00825	.0470 (2)	.0279 (4)
20	4.532	1	3	.263 (1)	-	.088 (2)	.081 (3)
21	13.956	11	26	.0295 (1)	.0067	.0274 (2)	.0170 (5)
23	21.558	50	10	.0995 (1)	-	.0267 (2)	.0236 (3)
24	19.518	31	33	.00527 (1)	.00393	.00463 (2)	.00355 (3)
25	3.470	⅓	1	.1180 (1)	.0523	.0857 (2)	.0650 (3)
Lymphatic leukemia							
3	31.440	147	14	.00905 (2)	-	.0103 (1)	.00624 (5)
17	8.446	5	5	.034 (5)	.049	.101 (1)	.071 (2)
19	23.649	31	21	.0437 (3)	-	.0535 (2)	lost
23	9.364	7	1	.0984 (4)	.0332	.173 (1)	.152 (2)
Leucosarcoma							
2	25.800	26	8	.0297 (4)	-	.0486 (2)	.0504 (1)
4	35.619	103	5	.0578 (1)	.0554	.0515 (3)	.0525 (2)
5	4.530	3	4	.0366 (3)	-	.0982 (1)	.0534 (2)
7	13.205	70	1	.145 (1)	.0025	.1097 (2)	.0664 (3)
9	17.256	29	8	.103 (4)	.165	.203 (1)	.180 (2)
11	10.088	4	2	.310 (3)	.374	.375 (1)	.350 (2)
12	6.858	4	3	.0504 (4)	.0126	.0827 (1)	.0636 (3)
15	9.560	6	7	.0520 (3)	-	.0570 (1)	.0443 (4)
Monocytic leukemia							
4	5.210	2	2	.120 (2)	.010	.124 (1)	.102 (4)
				.0915 (1)	-	.083 (3)	.086 (2)
6	4.964	1	4	.0620 (2)	-	.0535 (3)	.0640 (1)
7	4.504	½	1	.090 (2)	-	.104 (1)	.0727 (4)
Reticulum cell sarcoma							
2	15.081	18	10	.0190 (4)	-	.0501 (1)	.0422 (2)
Hodgkin's disease							
1	11.993	31	24	-	-	.0444	.008
Multiple myeloma							
3	8.660	7	1	.1688 (4)	-	.3442 (1)	.1949 (2)
4	5.002	2	4	-	-	.1020 (1)	.0660 (2)
5	10.908	16	61	.00268 (1)	-	.00109 (3)	.00119 (2)
Melanosarcoma							
1	7.748	4	5	.138 (1)	-	.0840 (3)	.0794 (4)

[a]Each patient is identified by the same number in this table and in all tables summarizing data for each disease.

cumulated for patients with neuroblastoma, seminoma, melanoma, Ewing's sarcoma, fibrosarcoma, osteogenic sarcoma, and carcinoma of the breast.[9, 37] The radiophosphorus was preferentially retained by the tumor or tumor-infiltrated tissue in one case of seminoma, one of melanoma, and in five of osteogenic sarcoma; in one case each of Ewing's sarcoma and of neuroblastoma, it was retained as well by the tumor as by liver and kidney; the primary tumor in six patients with carcinoma of the breast concentrated the radioactivity less well than did either normal or metastatic lymph nodes in the same patient. While there are these and unquestionably other exceptions, leukemic and neoplastic tissues in general preferentially concentrate radioactive phosphorus. This tendency, however, is apparently not great enough to make radioactive phosphorus a thor-

(EXPRESSED IN MICROCURIES PER GRAM OF WET TISSUE)

KIDNEY		MUSCLE		LYMPH NODE		BRAIN	LUNG	OTHER ORGANS	
.048	(3)	.023	(6)	.049	(2)				
.0113	(5)	.0079	(6)	.0238	(1)				
.152	(4)	.098	(5)	-				.040	(Blood)
.080	(3)	.032	(5)	-		.021			
.0305	(3)	.0216	(6)	.0278	(5)			.0059	(Blood)
.061	(5)	.020	(6)	.063	(4)				
.0139	(6)	.0178	(4)	.0243	(3)				
.0145	(4)	.00995	(5)	-		.0025			
.00275	(4)	.00237	(5)	-		.0021	.0665		
.560	(4)	.0308	(5)	-					
.00681	(4)	.00482	(6)	.00886	(3)	.00362			
.059	(3)	.043	(4)	-			.055		
.0342	(4)	.0270	(4)	.0547	(1)				
.0886	(5)	.0447	(6)	.135	(3)	.0184			
.0408	(3)	.0249	(5)	-		.0120		.0207	(Intestine)
.0371	(4)	.0141	(6)	.0338	(5)				
-		.0237	(4)	.0193	(5)	.0120			
.0498	(4)	.039	(5)	-					
.136	(3)	.091	(5)	-		.050			
.280	(4)	.0856	(6)	.180	(5)			.0864	(Blood)
.0654	(2)	.0400	(6)	.0420	(5)			.01625	(Blood)
.0434	(5)	.0190	(6)	.0559	(2)		.01875		
.090	(5)	.043	(6)	.116	(3)			.103	(Blood)
.0615	(4)	.0392	(6)	.0556	(5)				
.0332	(5)	.0263	(6)	.0474	(4)				
.0817	(3)	.0426	(5)	-					
.0357	(3)	.0077	(5)	-			.0211	.0603	(Tumor mass)
-		-		.000006					
.1846	(3)	.0656	(5)	-		.0087		.1087	(Tumor)
								.0310	(Intestine)
.0590	(3)	-		-					
.000754	(4)	.000745	(5)	-					
.0517	(5)	.0194	(6)	.0966	(2)		.0615	.114	(Tumor)

oughly satisfactory therapeutic agent. Enough of the material is deposited in normal tissues and cells to limit the total dose which can be given without toxic manifestations. Even in leukemia, enough P^{32} cannot be given to destroy all leukemic cells without at the same time destroying erythroid and megakaryocytic elements.[19]

The distribution of radioactive phosphorus in various organs at the time of death was determined in thirty-two of the fifty-three patients in our series who were examined at autopsy. The results of these determinations are shown in Table 1. Approximately 1 gram samples of the fresh tissues removed at autopsy were weighed on an analytical balance. Each sample was then transferred to a Kjeldahl flask and digested with concentrated sulfuric acid and 30 per cent hydrogen peroxide. The clear digest was diluted with distilled water

to a volume of 50 cubic centimeters and various aliquots of this solution were measured into the glass well of a plunger or dipping type Geiger counter (Bale tube) until a dilution was found which gave approximately the same number of counts per minute as did a standard radioactive phosphorus solution of known activity. From this determination the radioactivity in microcuries per gram of wet tissue was calculated. Some of these patients had received frequent injections of P^{32} up to within a few days of the time of death, whereas some had not received any therapy for several weeks. If the interval between the last injection of radioactive phosphorus and death was greater than nine weeks, the tissues were not assayed for radioactivity.

In Table 1, the figure in parentheses to the right of the activity in microcuries represents the relative activity of various tissues. In determining these relative activities, only six tissues were considered: bone marrow, liver, spleen, kidney, muscle, and lymph node. The rating (1) indicates greatest activity, whereas the rating (6) indicates least activity.

In nine of the ten cases of myelogenous leukemia studied, the bone marrow had a greater activity than any other organ assayed, while in only one instance did a lymph node have the greatest activity. In contrast to this, in chronic lymphatic leukemia, the liver, spleen, and lymph nodes had greater activity than the bone marrow in most cases. In every case of chronic lymphatic leukemia the bone marrow had a lower activity than the liver. In the eight cases of leucosarcoma, the figures are more variable, but here again the liver and spleen tended to have somewhat greater activity than the bone marrow. Curiously enough, in every instance except one where a lymph node was assayed, the activity of the lymph node was less than that of bone marrow, liver, spleen or kidney. This was probably due to the fact that none of these patients at the time of death had significantly enlarged lymph nodes, and it was frequently difficult to find a gram of lymph node free from fat and connnective tissue. Assays of the tissues from four patients with monocytic leukemia also gave variable results. The liver showed the greatest activity in two cases, the spleen in one, and the bone marrow in one. The one case of reticulum cell sarcoma studied showed more activity in the sarcomatous lymph nodes than in any other organ assayed. However, in the tissues of one patient with melanosarcoma, there was greater activity in the bone marrow than in the tumor.

The relative distribution of radioactive phosphorus among the different organs of the body depended, therefore, upon the type of neoplasm or leukemia and the degree of infiltration of any particular organ with abnormal cells. For example, at autopsy one patient with leucosarcoma showed considerable infiltration of the lungs with leukemic cells. On both gross and microscopic examination this infiltration was more marked at the left apex than at the left base. Assay of a portion of tissue from the left apex revealed an activity of 0.019 microcuries per gram of wet tissue, whereas tissue from the left base had an activity of only 0.015 microcuries per gram of wet tissue.

The distribution of radioactive phosphorus in the various tissues of patients with lymphatic leukemia indicates a somewhat less favorable differential uptake

of the radioactive material by the lymph nodes, spleen, and liver than was the case with the leukemic mice studied by Lawrence and his associates.[26] This may be due to the fact that our patients had received radioactive phosphorus for prolonged periods of time, whereas the mice were given a dose of P^{32} and the distribution in various tissues at 20, 40, 60, 80, and 100 hours was determined. The administration of P^{32} over longer periods of time may favor the accumulation of the material by those tissues which have a slower rate of phosphorus turnover.

D. *Absorption and Excretion of Radioactive Phosphorus.*—Absorption of P^{32} from the gastrointestinal tract of animals and of man has been studied by Chiewitz and Hevesy,[38, 39] Hevesy and associates,[40] Lawrence and co-workers,[1] Erf and associates,[5, 31, 41] and Cohn and Greenberg.[42] From 15 to 50 per cent of orally administered radioactive phosphorus is excreted in the urine and feces both in normal individuals and in patients with leukemia during the first four to six days. Most of this amount is lost in the stool and represents unabsorbed rather than excreted material. Absorption, however, is good enough so that radioactive phosphorus can be given therapeutically by mouth; this route of administration has been used in several studies. It has become the practice of most workers who give P^{32} orally to assume that 75 per cent of any given dose is absorbed.

When radioactive phosphorus is injected intravenously into patients with leukemia, from 5 to 25 per cent of the isotope is excreted, mostly in the urine, during the first four to six days.[31] Similar amounts are excreted by patients with polycythemia vera, but normal subjects regularly lose from 25 to 50 per cent within the same time period. After these first few days, the rate of excretion decreases in all subjects to less than 1 per cent of the injected dose per day. Erf and Lawrence suggest that the greater retention of intravenously administered P^{32} by patients with leukemia and polycythemia vera occurs because the isotope is more quickly fixed in the pathologic tissues and cells.

E. *Summary.*—Radioactive phosphorus emits beta-rays (electrons) which are capable of penetrating tissue to a depth of 0.7 cm. It differs from ordinary stable phosphorus only in that it is radioactive; its physiologic effects and its chemical properties are otherwise the same as those of the parent substance. Since this is so, however, relatively high concentrations of P^{32} in tissues and cells can be reached only if the amount of stable phosphorus given along with the isotope is kept at a minimum. The 14.3 day half-life of radioactive phosphorus permits steady irradiation of tissue for several weeks, yet is short enough so that radiation effects can be controlled.

Those tissues which have a high phosphorus content and which metabolize phosphorus rapidly selectively take up larger concentrations of radioactive phosphorus than do normal tissues. Since the radioactive isotope is built into nucleoprotein just as is ordinary P^{31}, those cells which are multiplying at the fastest rate use proportionately more of the P^{32} than do cells which are being produced more slowly. As a result, relatively high concentrations of radioactivity are reached in those organs principally involved in polycythemia vera, the leukemias, and the lymphomas. This preferential uptake, however, is not so great

that damage to normal tissues is avoided. In leukemia, for instance, one cannot give enough radioactive phosphorus to destroy all leukemic cells without also destroying erythroid and megakaryocytic elements.

When radioactive phosphorus is given by mouth, roughly 75 per cent is absorbed. After intravenous injection into patients, from 5 to 25 per cent is excreted within the first four to six days. The rate of excretion thereafter falls to less than 1 per cent per day.

III. POLYCYTHEMIA VERA

A. *Review of the Literature.*—There are seven papers[3, 4, 10, 12, 14, 15, 16] in the literature which desrcibe the clinical results obtained in the therapy of polycythemia vera with radioactive phosphorus. These papers are based on a study of forty-eight patients; for nineteen of these subjects specific clinical details are given either graphically or in case reports.

The first report was that of Lawrence[3] in 1940, who recorded data on two patients. The first of these was a 42-year-old woman, previously treated with phenylhydrazine, who had an erythrocyte level of 8,900,000 cells per cubic millimeter on the day treatment with radioactive phosphorus was started. She was given orally 5.3 millicuries of P^{32}, and seventeen days later a second oral dose of 7.0 millicuries was given. The first significant drop in the erythrocyte level occurred exactly one month after the first treatment; three months after the first dose the erythrocyte count was down to 4,800,000 cells per cubic millimeter. The patient was greatly improved symptomatically. The second patient was a 59-year-old woman who had previously been treated by repeated phlebotomies; as a result, the erythrocyte count was only 5,900,000 cells per cubic millimeter at the time P^{32} therapy was started. The first dose consisted of 5.2 millicuries of radioactive phosphorus, and the second dose, given twenty-four days later, of 7.9 millicuries. Both doses were apparently given orally, although this was not definitely stated. Following this therapy, the erythrocyte count first rose, due, presumably, to the discontinuance of phlebotomies, and it was not until two months after the first treatment that the erythrocyte count dropped below the original level. Three months after the first treatment, the red blood cell count was down to 4,000,000 cells per cubic millimeter. These first two reported cases are summarized in some detail because the results obtained in them parallel closely those observed in all subsequent studies.

Fitz-Hugh and Hodes[10] reported having treated eight patients with polycythemia vera, but no specific data regarding the dosage of radioactive phosphorus employed or the hematologic response obtained were given. The authors state that four of the eight patients were markedly improved both clinically and hematologically, one was unimproved, one was only slightly improved, and two had not been studied long enough to judge the effect. Two patients began to show hematologic relapse six and nine months, respectively, after the beginning of therapy. The authors further state that improvement may begin within two weeks of treatment, but full hematologic effect is rarely obtained until two to three months after therapy has been started.

Low-Beer, Lawrence, and Stone[12] mention that they have used P^{32} in the treatment of polycythemia vera for two and one-half years: fourteen patients were treated for a sufficient period of time to permit evaluation of the results. Data are given in this paper for only one patient, but the fourteen presumably include the two described in 1940 and the six for whom figures were recorded in a second report from the same laboratory.[4] The method of treatment was to give an initial dose of from 5.0 to 7.0 millicuries and to repeat it three to four weeks later. In those patients with simultaneous involvement of the leucopoietic system, an initial dose of from 4.0 to 5.0 millicuries was followed by biweekly additional amounts to keep up the initial level over a period of from three to five weeks. The total amount administered to six of the patients during the first seven to fifteen months of treatment varied from 12.3 to 48.8 millicuries; most of this was given by mouth.[4] The first signs of a satisfactory response were usually observed from six to eight weeks after treatment was started. Complete

or partial remissions were obtained in eleven of these fourteen patients, the remissions lasting from several months to two years.

Erf and Jones[14] gave detailed data on eleven patients with polycythemia vera treated with radioactive phosphorus. The polycythemia was probably secondary rather than primary in one of these patients. The material was injected intravenously; dosages varied largely because the authors were unable to obtain the isotope at regular or specified intervals of time, and no specific planned regime was followed. From 1.55 to 11.5 millicuries of radiophosphorus were given during the initial period of therapy, which ranged from 11 to 117 days. Additional amounts were administered parenterally to two of the patients after intervals of from eight to fourteen months. Gratifying clinical improvement occurred in the ten subjects with true polycythemia vera, but one of these died suddenly following a large gastric hemorrhage twelve weeks after initiation of therapy. A transient anemia (3.4 to 4.0 million cells) developed in six of the patients. It is not possible to draw conclusions about the length of remission induced by therapy since only three subjects had been followed for more than one year at the time of publication and five had been observed for four months or less.

Warren[15] treated three patients, two of whom were benefited. Data were recorded for only one patient, a 44-year-old woman with an initial erythrocyte count of 8,680,000 cells per cubic millimeter. She was given 3.0 millicuries of radioactive phosphorus intravenously, a second injection forty-six days later of 1.94 millicuries, and a third injection of 1.2 millicuries seventy-six days after the second injection. Seven months after the first treatment the red blood cell count was 4,520,000 and she was symptom-free. This complete remission lasted eleven months, after which interval she was given a fourth injection of 1.24 millicuries of P^{32}. Three years after the first course of therapy she still felt well.

Hall, Watkins, Hargraves, and Giffin[16] reported treating twelve patients with polycythemia vera. A satisfactory remission was observed in eight of these patients with complete relief of symptoms attributable to the polycythemia. The total dosage for a single course of therapy in these eight patients varied from 2.68 millicuries to 14.00 millicuries given in from one to three injections. The average dosage per course of treatment for these eight patients was 7.79 millicuries. The authors believe that the unsatisfactory therapeutic results obtained in the remaining four patients were due to inadequate dosage, even though from 6 to 14 millicuries were given in each instance. Even so, partial clinical improvement was noted in two of these individuals. Five of the twelve patients developed an anemia (3.4 to 4.0 million cells per cubic millimeter, from two to twenty-five months after the start of therapy; leucopenia (2,200 to 4,300 cells) also occurred in five subjects and thrombocytopenia (29,000 to 86,000 platelets per cubic millimeter) in four. Of particular interest is the fact that one patient, after a satisfactory remission of thirteen months' duration, died with the blood changes of acute leucopenic myelogenous leukemia.

From the foregoing summary of the literature, it is apparent that there is substantial agreement that excellent clinical and hematologic remissions can be obtained in the majority of patients having polycythemia vera by therapy with radioactive phosphorus. Very few of the reported cases, however, had been followed for a sufficiently long period of time to permit evaluation of the duration of remissions induced in this manner. Another feature which requires clarification is the proper dosage and method of administration, concerning which there is considerable diversity of opinion in published reports.

B. *Analysis of Results Obtained in Treating Thirty Patients With Polycythemia Vera.*—During the last four years, thirty patients with polycythemia vera have been treated with radioactive phosphorus in the Mallinckrodt Institute of Radiology. In addition to the routine blood and bone marrow studies, the following laboratory data were obtained on these patients: (1) x-ray of chest, (2) electrocardiogram, (3) circulation time, (4) basal metabolic rate, and (5) blood volume using Evans blue dye according to the method of Gibson and Evans,[43] as modified for use with the photoelectric colorimeter.[44] In those

TABLE 2. HEMATOLOGIC DATA ON THIRTY PATIENTS WITH

PATIENT	AGE AND SEX	TOTAL RADIOACTIVE PHOSPHORUS FOR EACH COURSE OF TREATMENT (MC.)	DURATION OF COURSE OF THERAPY (DAYS)	TIME FROM FIRST TREATMENT TO INITIAL DROP IN R.B.C. LEVEL (DAYS)	R.B.C. COUNT AT TIME THERAPY WAS BEGUN (MILLIONS PER C.MM.)
1	37, F	7.03	34	40	8.59
2	53, F	5.24	69	40	7.33
		16.88	429	34	6.66
3	45, M	7.41	112	30	6.34[a]
		1.80	1	27	6.35
		2.56	1		6.45
4	60, F	5.74[b]	2	30	7.83
5	42, M	5.74	66	88	9.00
6	38, M	8.44	70	41	6.65
7	56, F	13.25	75	33	7.00[a]
8	65, F	5.66	29	40	8.11
9	44, M	6.71	92	60	7.91
10	38, F	10.50	49	61	7.15[a]
11	50, F	4.90	43	28	7.91[a]
		2.53	32	28	5.75
12	23, F	7.78	139	30	7.56
13	47, F	6.92	120	47	6.47[a]
14	57, M	5.01	87	60	8.01
		3.62	56	112	6.80
15	66, F	5.17	2	48	7.57
16	59, M	4.07	1	58	7.98
17	56, M	4.03	1	41	6.02[a]
		3.00	21	30	6.38
18	63, M	4.14	44	83	5.78[a]
		3.58	40	92	5.87
19	56, F	7.72	286	--	5.88[a]
20	28, M	3.64	1	29	8.50[a]
21	65, M	9.36	338	--[c]	5.75[a]
22	59, M	3.78	44	44	7.65
23	49, M	4.79	65	--[c]	6.16[a]
24	61, M	3.96	1	--	8.18[a]
25	66, M	3.54	1	42	7.08
26	51, F	4.69	32	44	9.04
27	32, M	4.03	1	27	6.59[a]
28	63, M	3.50	1	--[c]	8.15[a]
29	33, M	3.44	1	21	7.09[a]
30	39, M	3.63	1	--	5.33[a]

[a] Patient treated by phlebotomies or phenylhydrazine up to the time P^{32} therapy was begun. It is probable that there was greater erythroid activity than was suggested by the red blood cell count.

[b] 7.66 millicuries given orally which is equivalent to 5.74 millicuries intravenously (assuming 75 per cent absorption from the gastrointestinal tract).

cases in which, after evaluation of all the above tests, there was still some doubt as to whether the patient had polycythemia vera or secondary polycythemia, arterial oxygen saturation was determined by the method of Van Slyke and Neill,[45] as described in Peter and Van Slyke's *Quantitative Clinical Chemistry Methods.*[46] It is felt that the diagnosis of polycythemia vera was conclusively established in every patient included in this series.

1. *Effect of Therapy on the Cellular Elements of the Blood:* Hematologic data on these patients are summarized in Table 2. A study of the figures in column 3 reveals that our patients were given smaller doses of P^{32} than have

POLYCYTHEMIA VERA TREATED WITH RADIOACTIVE PHOSPHORUS

LOWEST R.B.C. COUNT (MILLIONS PER C.MM.)	PLATELET COUNT AT TIME THERAPY WAS BEGUN (PER C.MM.)	LOWEST PLATELET COUNT AFTER THERAPY (PER C.MM.)	W.B.C. COUNT AT TIME THERAPY WAS BEGUN (PER C.MM.)	LOWEST W.B.C. COUNT AFTER THERAPY (PER C.MM.)	DURATION OF HEMATOLOGIC REMISSION (FROM TIME R.B.C. COUNT FELL BELOW 5.5 M. UNTIL IT AGAIN ROSE ABOVE 6.0 M.) OR INTERVAL BETWEEN COURSES OF TREATMENT (MO.)
3.29	2,490,000	358,000	15,450	3,400	26+
4.77	264,000	230,000	18,750	4,200	3[d]
4.81	839,000	517,000	6,750	17,300	
4.59	3,685,000	258,000	21,600	3,950	10
5.16	940,000	345,000	13,350	6,600	9
5.59	1,490,000	640,000	14,850	7,200	2+
3.35	2,036,000	29,000	43,700	1,100	30+
3.19	1,010,000	191,000	5,400	2,500	21+
4.75	458,000	454,000	6,400	3,200	3+
					(No further follow-up)
3.37	2,080,000	287,000	13,500	1,500	33+
2.97	1,233,000	107,000	11,100	1,200	25
4.77	1,266,000	626,000	15,750	7,050	6+
3.27	2,016,000	250,000	5,000	1,650	32+
4.34	1,818,000	164,000	11,600	4,100	19
4.30	1,250,000	469,000	10,300	3,650	2+
4.99	2,600,000	744,000	12,550	5,450	11
5.60	3,300,000	476,000	17,200	7,150	13+
5.12	820,000	364,000	9,900	4,300	5
4.46	490,000	392,000	7,700	3,800	9+
3.50	1,582,000	153,000	9,650	950	15+
4.47	1,607,000	191,000	10,400	1,550	15+
4.46	1,250,000	260,000	12,000	1,150	8
3.84	376,000	182,000	5,350	3,700	9+
3.54	1,543,000	724,000	13,300	5,250	10
5.09	740,000	--	11,850	7,200	3+
5.85	2,610,000	964,000	6,700	5,200	2+
3.70	1,802,000	343,000	12,250	7,600	12+
5.18	1,502,000	884,000	11,250	6,700	1+
3.95	--	274,000	7,100	3,950	7+
4.92	1,438,000	357,000	16,200	7,600	5+
5.15	2,160,000	1,590,000	14,200	7,800	1+
4.21	553,000	106,000	10,550	4,700	5
4.45	1,503,000	241,000	10,650	1,970	2+
4.65	1,931,000	715,000	9,250	6,300	2+
5.09	689,000	395,000	9,450	3,950	1+
6.60	790,000	482,000	15,650	7,750	1+
4.72	5,080,000	--	20,550	8,400	-

[c]Phlebotomies were done in addition to giving patient P^{32} therapy. No more than four phlebotomies were done in any patient, and these were all done within the first 1-2 weeks of therapy.

[d]Several months later the patient developed progressive anemia and leucocytosis. Died seven months later.

been advocated in other clinics (see footnote, page 110). In spite of this, the erythrocyte count after therapy fell below 4,000,000 cells per cubic millimeter in eleven of the thirty patients. In most of the other reports in the literature, the patients had not been followed for as long a period of time, or the data presented were insufficient to determine whether or not the patients were overtreated. However, as has already been emphasized, similar transient anemias were noted in patients treated by Erf and Jones[14] and by Hall and co-workers.[16]

The figures in column 5 reveal that in our cases the erythrocyte level did not begin to fall until three to six weeks after the initial injection was given. The

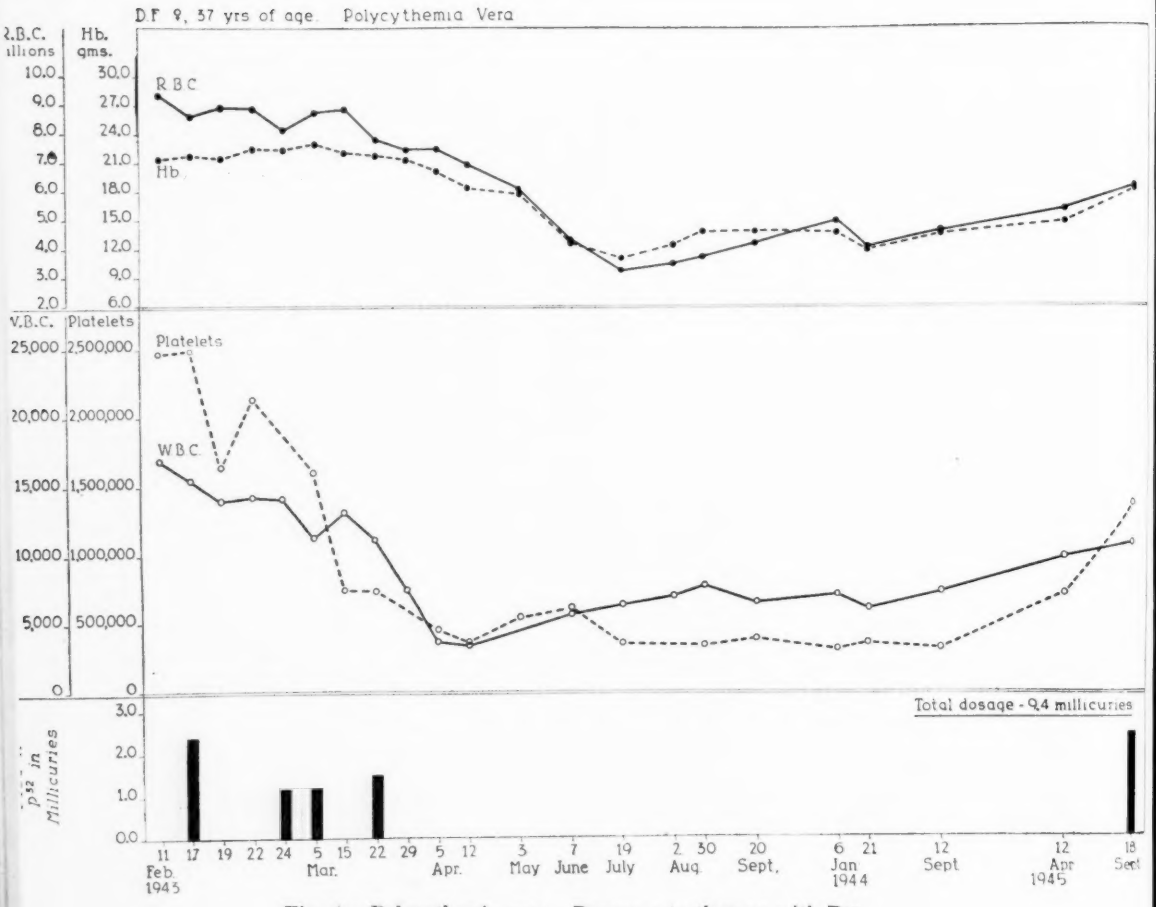

Fig. 4.—Polycythemia vera. Response to therapy with P^{32}.

Case Summary.—D. F., a housewife, 37 years of age, had had frequent dull headaches, often accompanied by dizzy spells, since 1935. She also complained of deep aching pains in the thighs and legs. Itching of the skin was severe at night and after bathing. During 1942 the headaches and dizziness increased in frequency and severity, and she began to tire easily. She had been "nervous" for many years.

Physical Examination (Feb. 11, 1943): Complexion was ruddy and there was marked cyanosis. The conjunctivae were injected. The heart and lungs were normal, and the blood pressure was 140/90. The spleen extended 6 cm. below the costal margin.

Laboratory Data: Blood counts (Feb. 11, 1943): erythrocytes, 9,360,000; hemoglobin, 21.3 grams; leucocytes, 16,850; reticulocytes, 2.8 per cent; platelets, 2,460,000; differential, normal. The total blood volume was 11,080 c.c. (173 c.c. per kilogram of body weight). Urinalysis, negative. The Kahn reaction was negative. The venous pressure and circulation time were normal. An electrocardiogram was normal except for left axis deviation. X-ray films of the chest and of the skull revealed no significant abnormalities.

Course: About a month after P^{32} therapy was started the patient began to feel better. This symptomatic improvement continued and within a few months she had no complaints except an occasional mild headache, rare episodes of pruritus, and spells of weakness and dizziness just before the menstrual periods. She continued to feel well until about September,

(Continued on opposite page.)

interval was much longer than this in some cases, but these were patients who received a relatively small initial dose or else lived so far from St. Louis that they could be followed only at infrequent intervals.

Erf's explanation[14] for this long latent period between the first injection of P^{32} and the first significant fall in the erythrocyte level certainly seems reasonable. He suggested that since human red blood cells apparently circulate for from 90 to 120 days, the effect on the total count of a slower rate of red cell delivery from the marrow would not become apparent for several weeks. This delayed fall in the erythrocyte level has several obvious practical disadvantages. If a patient's symptoms are severe, or the hematocrit value so high that some complication such as cerebral thrombosis or myocardial infarction is feared, it may not be possible or wise to wait for the full effect of P^{32} to become evident. These circumstances were encountered in three of our thirty patients, and on them phlebotomies were done; 500 cubic centimeters of blood were removed per day and no subject was bled more than four times. It is probable that this procedure would have been used more frequently during the first two weeks of P^{32} therapy if a determined effort had not been made to keep treatment as uncomplicated as possible.

As has been pointed out in previous publications,[16, 19] therapy with P^{32} as ordinarily employed in the treatment of polycythemia vera not only affects the erythrocyte level, but also has a profound effect on the platelet and leucocyte counts. The average platelet count of our polycythemic patients was 1,760,000 per cubic millimeter before therapy; this fell to an average minimum of 367,000 per cubic millimeter after the first course of treatment. In only one instance did the platelets fall below 50,000 per cubic millimeter. The average leucocyte count before treatment was 13,250 per cubic millimeter; after the first course of treatment, the average minimum count was 4,400 per cubic millimeter, but values as low as 950 were observed. In most cases in which a severe thrombocytopenia or leucopenia developed, this phase lasted only a few weeks. However, in general, the platelet and leucocyte levels have not returned to their original high values.

That the response to therapy varied greatly in different patients is apparent from Table 2. Patient 8 received 5.66 millicuries of P^{32} and the erythrocyte count dropped from 8,110,000 to 2,970,000 cells per cubic millimeter, whereas Patient 9 received 6.71 millicuries and the erythrocyte level dropped only from 7,910,000 to 4,770,000 cells per cubic millimeter. There are many other equally striking discrepancies in the hematologic response observed in different patients to a given dosage. *Consequently, therapy must be individualized to a high degree, and it is impossible to predict in advance how much therapy any given patient will require.*

1943, when she began to have severe hot flashes and increasing nervousness. Considerable illness among members of her family, the death of her father, domestic difficulties, and the fact that the patient was working as a clerk in a store, in addition to taking care of her home, tended to aggravate her symptoms. In general, during the following two years she felt considerably better than she had felt prior to P^{32} therapy. However, she continued to have hot flashes, nervousness, fatigability, and numerous minor complaints, all of which were thought to be on either a menopausal or psychogenic basis. Stilbestrol relieved some of these complaints temporarily.

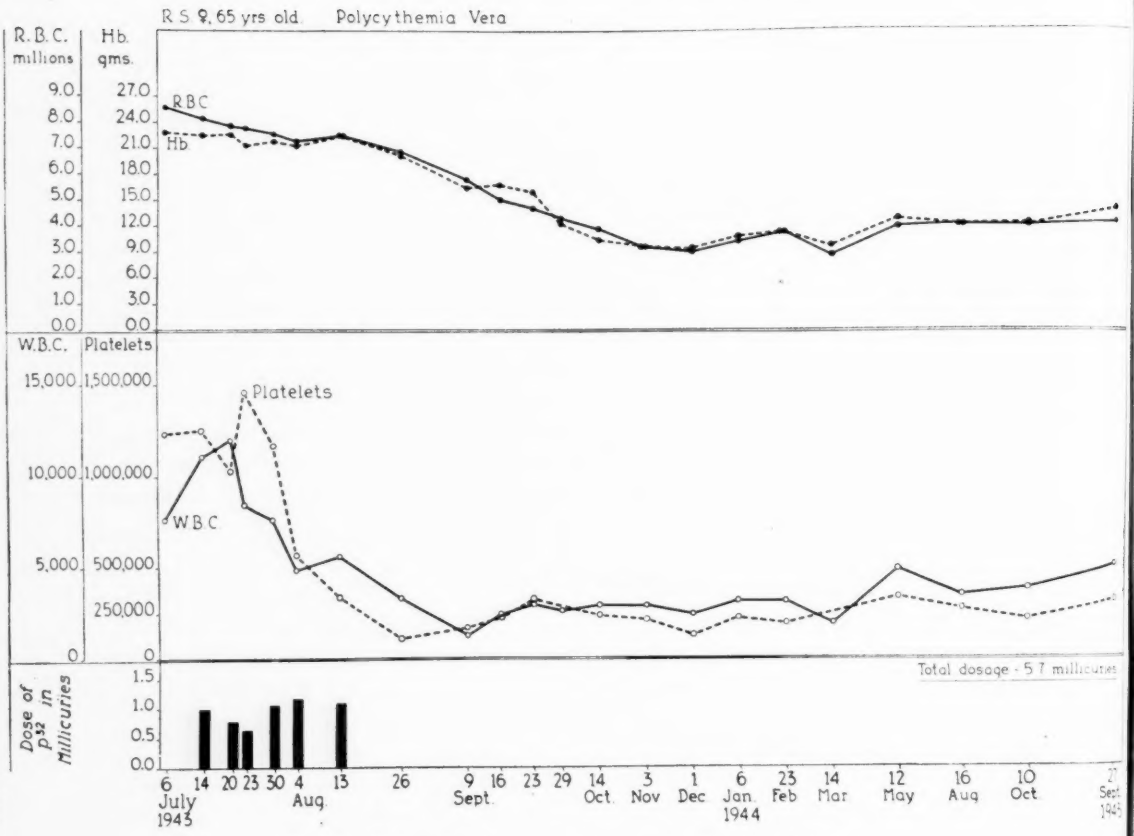

Fig. 5.—Polycythemia vera. Response to therapy with P^{32}.

Case Summary.—R. S., a widow, 65 years of age, suddenly developed numbness in the left hand in January, 1940; within a week this had progressed and involved the whole arm. Shortly therafter she began to have precordial aching pains, and occasional aching pain in her left shoulder. In January, 1941, she had pain in the left upper quadrant for the first time; her physician told her this was due to an enlarged spleen. About July, 1941, she began to tire very easily and felt drowsy much of the time. These symptoms persisted with little change until May, 1943, when she began to have severe pain in her feet, especially the toes, at night. By this time she had difficulty using her left hand because of numbness and stiffness. Itching after bathing was intense. Her weight decreased by nineteen pounds in two years' time.

Physical Examination: Blood pressure was 185/115. The complexion was very ruddy, and there was some cyanosis of the lips. Lymph nodes were not enlarged. The heart was enlarged. The spleen edge measured 18 cm. below the costal margin, and the liver edge 4 cm.

Laboratory Data: Blood counts (July 14, 1943): erythrocytes, 8,110,000; hemoglobin, 22.3 Gm.; leucocytes, 11,100; reticulocytes, 1.8 per cent; platelets, 1,230,000; differential: polymorphonuclear neutrophiles, 73 per cent; stabs, 13 per cent; juveniles, 2 per cent; basophiles, 1 per cent; lymphocytes, 5 per cent; monocytes, 6 per cent. The sternal bone marrow was extremely hyperplastic; the clumps of marrow were so dense that it was impossible to do an accurate count. Erythroid stimulation was marked, and myeloid cells and megakaryocytes were also present in increased numbers. Urinalysis was negative. The Kahn reaction was negative. Blood indices, coagulation time, and bleeding time were nor-

(Continued on opposite page.)

Detailed data for two patients are graphically illustrated in Figs. 4 and 5.

2. *Effect of Therapy on the Clinical Manifestations of Polycythemia Vera:* The clinical manifestations shown by these thirty patients, both before P^{32} was given and after the maximum benefit from therapy had been obtained, are summarized in Appendix Table 1. In every case, the patient felt greatly improved, at least temporarily, following therapy. However, in only eight patients were all symptoms completely relieved. This is probably attributable to the fact that many of the patients had disorders such as arteriosclerosis, hypertension, arthritis, menopausal syndrome, or psychoneurosis which accounted for some of their symptoms. Furthermore, the symptoms after therapy in many cases were much less troublesome than they formerly had been. In Table 3 are listed the more common symptoms of polycythemia and the percentage of our patients in whom each symptom was partially or completely relieved. It will be noted that headache is the only symptom which was not completely relieved in over 50 per cent of the patients. Of the 64 per cent of patients who still had headaches after therapy, the majority said their headaches had become mild and infrequent, and they mentioned them only when specifically questioned.

TABLE 3. THIRTY PATIENTS WITH POLYCYTHEMIA VERA—EFFECT OF P^{32} THERAPY ON SYMPTOMS

	NUMBER OF PATIENTS WITH SYMPTOM	% OF PATIENTS COMPLETELY RELIEVED	% OF PATIENTS PARTIALLY RELIEVED	% OF PATIENTS UNIMPROVED
Fatigability	20	65	30	5
Headaches	22	36	64	0
Dizziness	15	53	47	0
Aching pains in extremities	14	57	36	7
Itching	15	53	40	7
Blurring of vision	11	73	18	9
Burning of eyes and/or lacrimation	5	100	0	0

In Table 4 are recorded the abnormal physical findings encountered in these patients with polycythemia vera and the percentage of patients in whom there was a complete or partial disappearance of the abnormality following therapy with radioactive phosphorus. If the spleen was palpable, it was considered to be enlarged and is recorded as "splenomegaly"; if, after treatment, the spleen was no longer palpable, it is recorded as "abnormal finding completely dis-

mal. The total blood volume was 7,780 c.c. The basal metabolic rate was +15 per cent. An electrocardiogram showed right bundle branch block and auricular premature contractions. An x-ray of the chest revealed only moderate cardiac enlargement and aortic lengthening. An x-ray of the cervical spine revealed extensive osteoarthritic changes.

Course: About six weeks after P^{32} therapy was started the patient's strength began to improve and the itching cleared up entirely. The aching pains in the hands, shoulders, and over the precordium persisted. Six months later she had no symptoms except the pains (which were thought to be due to arthritis), and even these had lessened in intensity. She tired much less easily and was more alert. The spleen had decreased in size and now measured 9 cm. below the costal margin; the blood pressure had fallen to between 150/80 and 175/90. On Sept. 27, 1945, twenty-six months after the first treatment, she felt well except for aching pain in both hands and in the left shoulder. She had gained twenty-seven pounds in weight. The spleen measured 4 cm. below the costal margin.

TABLE 4. THIRTY PATIENTS WITH POLYCYTHEMIA VERA—CHANGES IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY

	NUMBER OF PATIENTS WHO HAD ABNORMAL PHYSICAL SIGN AT ONSET OF THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING COMPLETELY DISAPPEARED AFTER THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING PARTIALLY SUBSIDED	% OF PATIENTS IN WHOM THERE WAS NO IMPROVEMENT
Skin and/or mucous membranes bright red	25	96	4	0
Cyanosis	15	93	7	0
Conjunctivae red	9	100	0	0
Retinal venules distended	8	100	0	0
Petechiae and/or ecchymoses	2	100	0	0
Splenomegaly	28	68	32	0
Hepatomegaly	8	62.5	37.5	0
Systolic blood pressure 150 mm. Hg or higher	11	36.5	36.5	27

appeared," even though it is recognized that the spleen may never have returned to a perfectly normal size. Hepatomegaly is arbitrarily defined as that condition in which a liver edge was felt 2 cm. or more below the costal margin in the mid-clavicular line. No patient who had hepatomegaly was recorded as showing a complete disappearance of the abnormal finding after therapy unless the liver edge was no longer palpable after therapy. In all patients who had hepatomegaly or splenomegaly, measurements were routinely recorded with the patient breathing as quietly as possible. The measurements were taken from the costal margin to the lower border of the spleen or liver in the midclavicular line and in the anterior axillary line; in addition, the distance from the medial border of the spleen to the right or the left of the mid-line was recorded. If the spleen was only moderately enlarged, the only measurement recorded was the distance from the tip of the spleen to the costal margin at the maximum point. From these standardized measurements it was possible to follow changes in the size of these organs with reasonable accuracy.

The bright red color of the skin and mucous membranes, the cyanosis, the reddened conjunctivae, and the distended retinal venules disappeared after teratment in practically all cases. The only patient in whom the bright red color and the cyanosis did not completely disappear was a patient who was followed for only three months after therapy and his erythrocyte count had never dropped below 6,300,000 cells per cubic millimeter. Splenomegaly was present in all but two subjects, whereas hepatomegaly was present only eight times. All of the patients who had hepatomegaly also had a rather marked splenomegaly, the spleen edge measuring from 2 to 18 centimeters below the costal margin. About two-thirds of the enlarged spleens and two-thirds of the enlarged livers became nonpalpable after treatment.

It is interesting that eleven of the thirty patients had a systolic blood pressure of 150 or more millimeters of mercury, and eight of these eleven had a diastolic pressure of 100 or more millimeters of mercury. Of the abnormal physical findings, hypertension responded least favorably to P^{32} therapy.

3. *Duration of Remissions Produced:* Twenty-one of the thirty patients (Patients 1 to 5 and 7 to 22 in Table 2) have been followed for a year or more. In them, the duration of remissions has arbitrarily been defined as the interval from the time the red blood cell count fell below 5.5 million cells to that at which it again rose above the 6.0 million level. The longest remission so far observed is thirty-three months and is still continuing. In five patients, the remissions have been longer than two years; in eleven, longer than one year; and in seventeen, longer than nine months. Most of these subjects have not yet required a second course of therapy (last column, Fig. 2) so that no average figures can now be calculated. Additional radioactive phosphorus, as a matter of fact, has been given to only eight of the twenty-one patients. In seven instances, the duration of remissions was shorter than twelve months. One patient (Patient 2, Fig. 2), after a short remission of only three months, developed a progressive anemia, leucocytosis, and a leucocyte differential indistinguishable from that of subacute myelogenous leukemia. At the time of her death, more than 50 per cent of the leucocytes were myeloblasts or early myelocytes.

C. *Comparison of Radioactive Phosphorus With Other Forms of Therapy Used in the Treatment of Polycythemia Vera.*—Radioactive phosphorus unquestionably induces both clinical and hematologic remissions, often of long duration, in patients with polycythemia vera. Similar effects have been obtained with x-ray therapy or phenylhydrazine, and many clinicians have found therapeutic phlebotomy a satisfactory method of treatment. There is a growing conviction, however, which is shared by us, that administration of radioactive phosphorus is the treatment of choice for polycythemia vera. There can be no doubt that it is the most convenient method for the patient. He experiences no radiation sickness, does not have to take a drug whose dosage must constantly be regulated with care, does not have to subject himself to the inconvenience of repeated phlebotomies. Radioactive phosphorus exerts its therapeutic effect, as does x-radiation, by slowing the rate of erythrocytogenesis. Long clinical and hematologic remissions may be produced by one course of therapy.

It is not yet possible to conclude, however, that radioactive phosphorus prolongs life to a greater degree than do other forms of therapy. Polycythemia vera is a chronic disease of long duration in which spontaneous remissions not infrequently occur. The chronicity of the disease is well illustrated by the cases studied at the Mayo Clinic.[47] When Tinney, Hall, and Giffin[47] reviewed their experience with 163 patients seen prior to November, 1941, they found that in thirty-six the disease was of five or more years' duration. In nineteen patients, polycythemia had been present for ten or more years; in eight, for at least fifteen years; and in four, for at least twenty years. Death had occurred in twelve of these thirty-six patients from the following causes: myelogenous leukemia or leukemoid reaction, six; hemorrhage, three; thrombosis of the superior mesenteric vein, one; carcinoma of the stomach, one; and cirrhosis of the liver with Paget's disease of the skull, one. The most common cause of death among these patients, therefore, was myelogenous leukemia (or an anemia associated with a leukemoid reaction). The fact that one of the individuals treated by Hall and his associates[16] and one of our patients (Patient 2, Table 2) have

already died with similar manifestations suggests that radioactive phosphorus will not prevent this complication of polycythemia vera. An additional fifteen or twenty years of experience will be required before conclusions can be reached as to whether P^{32} will prolong life more than have x-ray therapy, phenylhydrazine, and phlebotomy.

Even though a number of the remissions obtained by other investigators and by us are now measurable in years and are still in progress, there is little reason to believe that cures have been produced in these instances. The number of observed relapses is great enough to make one believe that relapses will also eventually occur in these persons.

Emphasis has already been given to the fact that complications may result from therapy with P^{32} as from other forms of treatment. These are anemia, leucopenia, thrombocytopenia, and possibly aplastic anemia. Prevention of these complications can be accomplished at the present time only by avoiding overdosage. Because of difference in susceptibility of patients to the isotope, the dosage must be individualized to a high degree. The chief practical disadvantages associated with the use of radioactive phosphorus at the present time are (1) the moderately high cost of producing it, (2) its relative scarcity, and (3) the relatively long latent period (from thirty to sixty days) which occurs before the red blood cell count begins to fall.

D. *Summary and Recommended Method for Administering P^{32} to Patients With Polycythemia Vera.*—From the results reported in the literature and from those here recorded, it is clear that the administration of radioactive phosphorus to patients with polycythemia vera induces satisfactory clinical and hematologic remissions which not infrequently last for two or more years. The fall in the red blood cell count, however, usually does not begin for from thirty to sixty days; for this reason, if symptoms are severe, it is often advisable to reduce the red cell level by removing blood during this period. Treatment with P^{32} seems superior to other methods used in the past, but it is not yet certain that this form of therapy will prolong life to a greater degree than will x-radiation, phenylhydrazine, or repeated phlebotomies. Dosage must be individualized and carefully controlled if the production of anemia, leucopenia, and thrombocytopenia is to be avoided.

As a result of the experience accumulated in this clinic, radioactive phosphorus is now given to patients with polycythemia vera according to the following routine. After the diagnosis has been established, from 3.5 to 4.0 millicuries are injected intravenously. The height of the red blood cell count and the weight of the patient are used as a guide to decide which of these doses will be employed (see footnote, page 110). No additional P^{32} is given for three months. If symptoms are severe, however, or the erythrocyte level is so high that complications such as thrombosis are feared, 500 cubic centimeters of blood are removed daily until the red cell count has been reduced to between 6 and 6.5 million cells. This level is maintained by additional bleedings during the first two to four weeks. They are then discontinued, if possible, so that the effectiveness of the P^{32} may more easily be evaluated. If the count remains above 6.0

million cells, a second injection of from 1.0 to 3.0 millicuries is given about ninety days after the first injection. Additional phlebotomies are usually not required but are done if the erythrocyte level rises high enough so that symptoms reappear. Many patients do not require this second injection; a few must be given a third injection of from 1.0 to 3.0 millicuries after a second interval of ninety days. By this time a remission has almost invariably been produced and the patient is observed at intervals of from one to two months either by us or by his own physician. No additional therapy is given until a relapse occurs. This schedule is more conservative than that used by most other clinicians who have used radioactive phosphorus therapeutically but has been adopted because it rarely causes any of the cellular elements of the blood to fall significantly below normal levels.

IV. MYELOGENOUS LEUKEMIA

A. *Review of the Literature.*—There are nine reports in the literature dealing with the therapeutic effectiveness of radioactive phosphorus in myelogenous leukemia.[1-3, 5, 7, 9, 10, 12, 15] These reports are based on the study of 107 patients of whom eighty-two had chronic myelogenous leukemia, twenty had acute myelogenous leukemia, and five had subacute myelogenous leukemia.

Lawrence[1] presented detailed data on two patients. The first was a 52-year-old man with chronic myelogenous leukemia. This man was given 86 millicuries of radioactive phosphorus by mouth during a period of seventy-two days. Throughout this time there was progressive symptomatic improvement. The total leucocyte count dropped from 600,000 cells per cubic millimeter to within the normal range; all myeloblasts and myelocytes disappeared from the blood; and the erythrocytes rose from 3,350,000 cells per cubic millimeter to normal values. There was a gradual and progressive decrease in the size of the liver and spleen until neither was palpable. The patient remained symptom-free for a period of about four months, at the end of which time the leucocyte, erythrocyte, and thrombocyte levels all decreased to low levels and hemorrhagic manifestations appeared. Shortly thereafter, the leucocyte count rose progressively, and the liver and spleen enlarged. Severe cough, fever, and night sweats developed, and cellulitis of the face occurred terminally. At post-mortem examination, miliary tuberculosis was found; there was a mild amount of myeloid hyperplasia in the bone marrow and slight myeloid metaplasia in the spleen and liver.

The second patient was a woman, 40 years of age, who had acute myelogenous leukemia. She was given iron, vitamins, liver extracts, and small doses of x-ray therapy; the total leucocyte count dropped temporarily following each x-ray treatment, but there was no clinical improvement. The patient was then given two oral doses of radioactive phosphorus four weeks apart (4.77 and 1.19 millicuries, respectively). The leucocyte count fell to leucopenic levels, but again there was no clinical improvement. At death, post-mortem examination revealed pathologic changes of acute myelogenous leukemia without histologic evidence of irradiation effects.

Since this original report by Lawrence, specific data have been published on fifty-three additional cases. Forty-three previously unreported cases were analyzed in a comprehensive paper by Erf, Tuttle, and Lawrence.[5] It is obviously impossible to summarize each of these cases here, and only their general conclusions will be discussed. All of the other detailed case reports in the literature, ten in number, are summarized in Table 5.

Erf, Tuttle, and Lawrence[5] tabluated the symptoms and physical signs before and after P^{32} therapy of thirty-eight patients with chronic myelogenous leukemia and eight patients with acute myelogenous leukemia. The total dosage of radioactive phosphorus given to the patients with chronic myelogenous leukemia ranged from 2.3 to 90.7 millicuries, with an average total dosage of 26.5 millicuries. Among the patients with acute myelogenous leukemia, the total dosage varied from 2.0 to 24.1 millicuries, with an average of 10.4. The hematologic

TABLE 5. SUMMARY OF REPORTS FROM THE LITERATURE ON PATIENTS WITH MYELOGENOUS LEUKEMIA TREATED WITH P^{32}

AUTHOR AND REFERENCE	PATIENT	AGE AND SEX	APPROX. INITIAL W.B.C. COUNT (PER C.MM.)	TREATMENT	COMMENTS
Warren[2]	1	35, M	41,200 (50 per cent "blasts")	4 injections totaling 5.9 mc. over period of 6 weeks; 4 blood transfusions also given	Subacute myelogenous leukemia; during period of therapy W.B.C. fluctuated widely, rising terminally to 97,500 cells per c.mm. with more than 70% blasts; patient died about 44 days after start of P^{32} therapy
Lawrence[3]	1	58, F	22,000	5 doses given orally over period of 10½ months (total, 16.22 mc.)	Each treatment was followed by restoration of approximately normal leucocyte level and disappearance of leukemic skin rash; patient still living and in excellent clinical condition 13 months after onset of therapy
	2	29, M	132,000	3 doses given orally over period of 7 months (total, 10.74 mc.)	Following treatment, leucocyte count gradually dropped and erythrocyte level rose to approximately normal; shortly after first dose of P^{32} he improved clinically and 1 year later was still symptom-free; liver and spleen, which were initially greatly enlarged, became no longer palpable
	3	39, F	155,000	5 doses given orally over period of 2 months (total, 21.00 mc.)	Following therapy leucocyte count dropped to 8,000 and then fluctuated between 10,000 and 20,000 for next 6 months; erythrocyte count rose to normal; splenomegaly and hepatomegaly disappeared
Kenney[9]	1	25, F	307,200	8 doses (? route) totaling 18.9 mc. over period of 5½ weeks	3 months after start of P^{32} therapy W.B.C. count was 12,000 and spleen had decreased markedly in size; 1 month later W.B.C. count began to rise rapidly; this continued in spite of x-ray therapy; died 4½ months after onset of P^{32} therapy
	2	41, M	310,000	23 doses (? route) totaling 50.8 mc. over period of 15 months	Leucocyte count fairly well controlled by treatment; erythrocyte level rose from 3,400,000 to around 4,500,000; patient still living at time of report
Low-Beer and associates[12]	1	12, F	380,500	14 doses totaling 16.038 mc. given over period of 12 weeks; 3 doses (about 1 mc. each) given during next 5 months; additional 7 doses totaling 4.13 mc. during last 6 weeks of life	14 weeks after onset of P^{32} therapy W.B.C. count was 10,300, R.B.C. had risen from 2,070,000 to 5,180,000, spleen, which originally filled most of the abdomen, was barely palpable; patient gained 20 pounds, felt well; fairly complete remission lasted about 4 months, but death occurred 10 months after onset of therapy

TABLE 5—CONT'D

AUTHOR AND REFERENCE	PATIENT	AGE AND SEX	APPROX. INITIAL W.B.C. COUNT (PER C.MM.)	TREATMENT	COMMENTS
Low-Beer and associates[12]	2	44, F	342,400	P^{32} given orally; exact dosage not given, but radiation level was built up to a 5.0 mc. level	Within 6 weeks leucocyte count dropped to normal and erythrocyte count rose to normal; previously palpable lymph nodes disappeared and patient gained 10 pounds; patient remained in excellent condition with very slight fluctuations of blood counts for 8 months; two subsequent relapses were treated with courses of P^{32} therapy; at time case was reported, patient was again in excellent remission (11 months after onset of therapy)
Warren[15]	1	45, F	322,000	8 i.v. injections totaling 20.41 mc. given between 11/20/40 and 4/6/42	Patient improved clinically and spleen became smaller; following initial drop in W.B.C. count, leucocyte level fluctuated between 14,400 and 44,000 until terminally, when it rose to 320,000; during first few months of therapy, R.B.C. count rose slightly; patient expired 17 months after onset of therapy
	2	60, F	250,000	14 doses (all but 4 i.v.) totaling 29.62 mc. between 4/17/42 and 6/4/43	This patient had failed to respond to x-ray therapy and was virtually moribund when P^{32} was started; she improved clinically and spleen decreased to two-thirds its former size; W.B.C. count never dropped below 80,000; death occurred 19 months after onset of therapy

changes during and after treatment were briefly summarized for ten of the patients with chronic myelogenous leukemia. Apparently, three of these had previously been reported by Lawrence and his associates. None of the patients with acute leukemia was benefited by therapy, and all were dead at the time of the report. Of the thirty-eight chronic cases, eleven of the patients had partial remissions and five had complete remissions; twenty-one had died. Two patients had essentially complete remissions which were of nearly two years' duration at the time of the analysis. Of the thirty-eight chronic cases, twelve of the patients had had no therapy previous to P^{32}. The response to P^{32} was less satisfactory in those who had had x-radiation previously. As the duration of the disease previous to the administration of P^{32} was quite similar in the two groups, these investigators concluded that previous x-radiation reduces the effectiveness of radiophosphorus. In those cases examined at autopsy, the gross findings were those usually described for leukemia. The histopathology was interesting in that there were no cellular changes in normal cells that could be attributed to local irradiation caused by the radiophosphorus; many sections did show cellular lysis or rhexis in leukemic cells. The authors concluded that in the doses they employed, radiophosphorus produced no morphologic damage to normal tissues.

Craver[7] summarized the results obtained in the treatment of eleven adults with chronic myelogenous leukemia. No specific data concerning the dosage of P^{32} employed or changes in the blood counts were given for any of these patients. Only one was followed for as long as one year. Three of the eleven patients died 20.5, 4, and 4 months, respectively, following the

beginning of P^{32} therapy, whereas eight were still living at the time of the report. Craver concluded that of the eleven patients, "seven, including the one who died after 20.5 months, showed definite evidence that P^{32} is an effective therapeutic agent in this type of leukemia." Craver also mentions that one patient with acute myelogenous leukemia and one with subacute myelogenous leukemia were treated. One of these died in one week and the other in six weeks; in neither patient was there any detectable effect of P^{32}, favorable or unfavorable. No details were given.

Kenney[9] reported having treated six patients with chronic myelogenous leukemia, and one each with acute and subacuté myelogenous leukemia. Case reports were given for only two patients; data for these two are summarized in Table 5. The patient in the acute phase of the disease died one week after the administration of 1.5 millicuries of P^{32}. The patient who was in the subacute phase of the disease received three doses of 0.8 millicurie each at weekly intervals; death occurred six weeks after the first treatment. In all six individuals with chronic myelogenous leukemia, the following effects were observed: the leucocyte count was reduced to approximately normal levels; the percentage of myeloblasts and myelocytes in the bone marrow was reduced; the erythrocyte counts and hemoglobin levels rose during therapy; enlarged spleens decreased in size; and in no case was radiation sickness observed.

Fitz-Hugh and Hodes[10] reported having treated five patients with chronic myelogenous leukemia, but no data were given concerning any of them. The authors state that one patient obtained an excellent remission which lasted several months, and a second patient who was then under treatment seemed to be responding satisfactorily. The other three persons were in the late stages of the disease and were unimproved by treatment.

Nineteen patients with chronic myelogenous leukemia, two with subacute myelogenous leukemia, and nine with acute myelogenous leukemia were treated with radioactive phosphorus by Warren.[15] Only two case reports were given. These are summarized in Table 5. Warren stated that nine of the nineteen patients with chronic myelogenous leukemia were "helped" by therapy, both cases of subacute myelogenous leukemia were "helped," but none of the acute cases was benefited. Those patients were considered helped who "showed definite clinical and laboratory evidence of improvement."

There is substantial agreement among all these investigators that patients with acute myelogenous leukemia do not respond favorably to therapy with P^{32}. There is also agreement that in chronic myelogenous leukemia administration of radioactive phosphorus will usually restore the leucocyte count to normal or approximately normal levels, and that a rise in the erythrocyte level frequently follows. This hematologic improvement is quite consistently accompanied by definite symptomatic improvement. As in polycythemia vera, adequate data are given for so few cases of myelogenous leukemia that it is not possible to evaluate the duration of the remissions induced or to compare the results of P^{32} therapy with the results which have been obtained with other forms of treatment such as x-ray. Detailed data are given in the following section for all of our patients in the hope that they may provide at least tentative answers to these important problems.

B. *Analysis of Results Obtained in Treating Thirty-Nine Patients With Myelogenous Leukemia.*—Thirty-nine patients with myelogenous leukemia have been treated with radioactive phosphorus at the Edward Mallinckrodt Institute of Radiology. Twenty-one of these have died, and eighteen are still living. During the first ten days to two weeks of therapy, each patient was seen once every two or three days. Thereafter, the interval was gradually lengthened as the disease was brought under control, but even during protracted intervals of complete freedom from symptoms, each patient was seen at least once every two months. A sternal bone marrow examination was done in every case immediately before therapy was begun and at variable intervals thereafter depending on the severity and progress of the disease. A total of from two to four sternal aspirations have been performed on most of the patients who have been

under observation for two years or longer. The basal metabolic rate was also followed in most instances.

It is important to emphasize that there was no selection of patients for this series. Only those who lived so far away that they could not be seen at frequent intervals were rejected and treated with x-radiation or Fowler's solution. Many of the thirty-nine persons to whom P^{32} was given had been treated for years by their family physicians and were referred to us only when previously employed therapeutic measures no longer seemed effective. The average duration of symptoms at the time P^{32} therapy was started was slightly over two years, and ten of the patients had had symptoms for three years or longer. At least nine patients (Patients 1, 6, 16, 19, 20, 21, 25, 28, and 38) were either considered to have subacute myelogenous leukemia or to be in the terminal stage of chronic myelogenous leukemia.

1. *Effect of Therapy on the Cellular Elements of the Blood:* The hematologic and various other data on these patients are summarized in Table 6. Twenty-one of the subjects were men, and eighteen were women. Twenty-four of them had had some other form of treatment before P^{32} was first administered, including x-radiation in twenty cases. In very few instances, however, had any other therapy been given during the last four months prior to the first injection of P^{32}. The initial leucocyte count was elevated in all thirty-nine patients, and there were no examples of subleukemic leukemia in this series. Thirty-four of the thirty-nine patients had an anemia of less than 4,000,000 cells per cubic millimeter, and seven had a thrombocytopenia of less than 300,000 platelets per cubic millimeter (this is definitely below the normal range by Dameshek's method).

The wide variation in the dosage of radioactive phosphorus required to restore the leucocyte count to approximately normal levels needs to be emphasized. In eight patients less than 6 millicuries sufficed to reduce the white blood cell count to 15,000 or less, while two patients received in excess of 50 millicuries without ever showing a drop of the leucocyte count to this level. In nineteen of the patients the dosage required to lower the leucocyte count to less than 15,000 cells per cubic millimeter was between 6 and 12 millicuries; the time required varied from 15 to 120 days. The height of the initial leucocyte count was not the only determining factor for either the total amount of P^{32} or the time required to accomplish this result.

As the leucocyte count decreased, the percentage of polymorphonuclear leucocytes increased and the percentage of myelocytes and myeloblasts decreased significantly in every case except one. This one patient (Patient 25) was in the terminal stage of the disease and died nine days after treatment was begun. The erythrocyte level increased by more than half a million cells per cubic millimeter in twenty-five of the thirty-three patients who received no transfusions; in twelve instances the increase amounted to more than 1,000,000 cells per cubic millimeter. The platelet count was less than 300,000 per cubic millimeter at the time P^{32} therapy was started in seven patients; in four, the thrombocyte level rose after treatment to above 900,000 per cubic millimeter. Bone marrow examinations were usually repeated after the blood counts had been

TABLE 6. HEMATOLOGIC DATA ON THIRTY-NINE

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	BLOOD COUNTS AT TIME P32 THERAPY WAS STARTED						P32 THERAPY REQUIRED TO RESTORE W.B.C. COUNT TO APPROX. NORMAL (4,000-15,000)		MOST NORMAL BLOOD COUNTS AFTER P32 THERAPY					
			W.B.C. (PER C.MM.)	R.B.C. (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% POLYMORPHONUCLEAR NEUTROPHILES	% MYELOCYTES + MYELOBLASTS	MC.	DAYS	W.B.C.	R.B.C.[b]	HG (GM.)	PLATELETS	% POLYMORPHONUCLEAR NEUTROPHILES	% MYELOCYTES + MYELOBLASTS
1	38, M	X-R	135,000	3.59	11.6	164,000	1	43	19.744	69	11,000	4.44	14.3	1,830,000	48	6
2	25, F	Fo	155,000	3.69	10.8	1,180,000	17	33	9.537	33	8,700	4.45	12.5	1,436,000	82	0
3	58, M	X-R	111,000	5.51	15.1	1,664,000	26	23	9.704	47	8,100	5.42	14.7	1,471,000	75	0
4	42, M	X-R, Fo	72,000	3.26	8.7	593,000	14	11	7.415	18	7,850	3.56	8.9	1,120,000	57	3
5	39, F	X-R	107,000	3.78	12.3	3,553,000	30	29	4.712	24	8,500	4.64	13.5	3,544,000	76	0
6	41, M	None	497,000	2.52	7.4	25,000	27.5	62.5	1.251	23	7,150	4.31 T-1	10.9	1,100,000	62	5
7	67, F	X-R	464,000	2.25	7.3	46,000	15	44	5.780	63	7,600	4.63	13.0	1,150,000	70	0
8	50, F	X-R, Fo	115,000	2.35	7.5	921,000	35	28	9.589	37	7,500	3.14 T-3	9.6	1,320,000	78	0
9	57, M	X-R	22,000	5.05	16.5	1,179,000	43	10	3.470	22	7,400	5.61	17.5	1,390,000	75	0
10	37, M	X-R, Fo, T.	144,000	3.88	9.4	2,663,000	40	34	69.863[c]	539	16,300	5.10	11.0	1,460,000	45	18
11	43, F	None	344,000	2.96	8.6	444,000	21	29	16.838	119	8,750	4.43	12.9	1,287,000	60	4
12	58, F	X-R	288,000	2.83	6.5	1,560,000	3	52	25.324	190	8,050	3.57 T-4	9.0	1,150,000	76	0
13	48, F	X-R	164,000	2.90	7.9	487,000	12	37	20.128	124	7,850	4.44	12.5	1,509,000	77	3
14	37, F	None	300,000	2.94	8.0	1,117,000	18	34	19.493	143	6,750	5.27	13.9	1,420,000	68	3
15	31, M	None	123,000	3.23	10.8	652,000	34	25	9.583	70	6,400	5.10	14.7	1,348,000	68	1
16	26, M	X-R	25,450	4.48	14.4	591,000	37	9	5.264	30	7,800	4.12	12.5	870,000	69	1
17	35, F	None	175,000	3.48	11.5	557,000	43	14	6.200	80	8,200	4.58	15.4	1,540,000	62	3
18	13, M	None	502,000	1.93	6.4	559,000	13	35	8.806	120	8,300	5.76	13.8	1,300,000	66	3
19	31, M	X-R	227,000	2.07	4.7	200,00	34	49	5.631	30	8,450	3.57 T-15	11.0	485,000	49	4
20	27, M	X-R	139,000	2.05	6.5	185,000	27	42	4.532[c]	9	89,000	2.62 T-3	6.3	225,000	31	50
21	52, M	X-R	429,000	2.08	6.2	360,000	6	50	13.956[c]	81	35,250	2.65	9.3	620,000	26	21
22	47, M	Fo	134,000	3.74	12.5	740,000	36	37	6.756	34	7,500	5.27	14.9	1,200,000	71	2
23	64, M	Fo, X-R	389,000	2.72	9.4	1,314,000	20	27	11.758	43	7,450	5.19 T-2	14.1	1,313,000	70	0
24	63, F	None	109,000	3.17	9.8	1,376,000	16	32	8.245	38	8,100	3.89	11.3	1,487,000	56	5
25	41, M	X-R	77,000	1.60	4.9	371,000	28	16	1.770[c]	3	56,500	1.08	3.9	194,000	28	23
26	42, M	X-R	53,000	6.14	15.1	602,000	50	8	5.063	17	8,900	5.67	15.4	1,531,000	81	1
27	16, M	Fo, B.M.	34,050	5.37	15.0	1,058,000	30	24	8.249	71	10,600	3.99	13.3	733,000	74	0
28	78, F	None	492,000	2.70	7.4	842,000	6	41	3.270[c]	5	443,000	1.46	4.4	510,000	20	20
29	51, F	None	243,000	3.32	7.0	1,477,000	14.5	44.5	7.315	30	8,000	5.00	14.2	1,540,000	76	0
30	36, F	None	550,000	2.10	6.8	270,000	7	45	54.590[c]	400	22,350	4.53	11.5	936,000	47	19
31	22, F	None	110,000	3.81	11.9	2,095,000	27	23	7.825	29	8,700	4.52	13.5	1,540,000	81	0
32	39, M	X-R	83,000	3.36	10.5	410,000	27	16	7.229	67	14,900	5.40	15.4	1,510,000	46	2
33	57, M	X-R, Fo	53,000	3.40	10.8	1,394,000	20	43	9.035	69	14,800	4.65	13.3	1,620,000	55	6
34	22, F	T	244,000	2.01	5.6	320,000	13	28	8.401	35	7,300	3.42 T-2	9.0	1,300,000	78	2
35	46, F	None	258,000	3.58	10.9	805,000	31	15	8.515	85	7,200	4.49	14.2	900,000	63	0
36	31, F	X-R	64,000	3.60	11.7	790,000	36	30	8.056	15	7,000	4.56	13.5	920,000	71	0
37	45, M	None	228,000	3.99	9.8	1,080,000	26	16	15.513[c]	132	20,450	4.75	13.8	1,050,000	60	5
38	76, M	None	56,500	1.96	6.4	85,000	23	37	4.256	10	6,250	2.64	7.3	120,000	69	0
39	38, F	None	132,000	3.42	9.2	1,265,000	23	31	6.59	47	10,950	4.76	13.0	1,590,000	81	2

[a] X-R = X-ray therapy; Fo = Fowler's solution; B.M. = bone marrow extract; T = transfusions.
[b] T = Transfusions. The figure indicates the number of transfusions given.

PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

DURATION FROM RESTORATION OF NORMAL W.B.C. COUNT UNTIL DEATH OR 9/1/45 (MO.)	TREATMENT AND BLOOD COUNTS DURING FIRST YEAR OR FRACTION THEREOF FOLLOWING RESTORATION OF LEUCOCYTE COUNT TO APPROXIMATELY NORMAL (4,000-15,000)			TREATMENT AND BLOOD COUNTS DURING SECOND YEAR OR FRACTION THEREOF FOLLOWING INITIAL RESTORATION OF LEUCOCYTE COUNT TO APPROX. NORMAL			TREATMENT AND BLOOD COUNTS DURING THIRD YEAR FOLLOWING INITIAL RESTORATION OF LEUCOCYTE COUNT TO NORMAL			DURATION OF DISEASE (MO.) FROM FIRST SYMPTOM	
	MC. OF P32	W.B.C. RANGE	R.B.C. RANGE[b]	MC. OF P32	W.B.C. RANGE	R.B.C. RANGE[b]	MC. OF P32	W.B.C. RANGE	R.B.C. RANGE[b]	TO START P32	TO DEATH OR 9/1/45
6	15.084	11,100-358,000	4.44-1.09 T-3							18	26
10	17.502	1,650-152,000	4.45-2.33 T-6							48	59
38+	1.548	1,600- 22,650	3.56-5.66	1.654	10,350-19,450	4.49-5.80	None	9,800-18,200	5.13-5.95	15	54+
3	3.666	7,850-184,000[d]	3.56-2.79							36	40
17	8.839	8,150- 26,450	4.63-3.13	9.080	12,700-28,650	4.54-1.64 T-3				26	64
4½	8.871	7,150-383,000	2.19-4.31 T-4							36	41
35+	2.965	5,400- 25,800	3.26-4.69	5.095	6,150-114,000	3.42-4.63	5.994	6,650-31,800	3.93-4.64	36	73+
2	None	3,800- 17,500	1.62-3.14 T-13							60	63
36+	6.5	6,400- 15,150	4.11-6.20	2.150	9,700- 15,600	5.15-7.14	2.088	13,250-16,000	5.88-6.76	27	64+
-										39	59
17	18.232	8,750-150,000	4.43-1.38 T-15	2.564	2,500- 48,500	0.87-2.42 T-8				9	31
12	1.465	1,550- 32,550	3.57-1.32 T-6							11	30
8	24.829	7,850-221,000	4.44-2.27							54	66
9	24.743	6,550- 48,000	2.05-5.76							30	44
26+	7.095	6,400- 21,000	3.62-5.10	11.991	11,800- 30,350	4.05-5.10	1.026	24,200-31,800	4.10-3.84	6	34+
2	0.676	1,950- 11,200	4.12-1.63							4	7
21+	8.593	7,650- 46,500	3.84-5.01	5.482	7,500- 45,100	4.18-4.87				4	28+
5½	19.965	8,300-120,000	5.76-2.30							18	27½
4½	12.686	32,400- 900	3.57-0.93 T-12							10	15½
-										24	24½
-										21	24
17+	18.864	3,500- 67,500	5.27-3.69	4.960	7,500- 65,000	4.32-4.98				24	42+
10½	9.800	1,850- 51,500	2.80-5.19 T-1							60	72
7	11.273	8,100- 60,500	3.89-1.96 T-6							12	20
-										62	62 1/10
14+	15.262	7,800- 55,500	4.76-6.10	6.235	12,250- 47,000	5.32-5.58				6	21+
8	11.717	7,350-100,000	4.22-1.54 T-14							10	20
-										96	96 1/6
13+	7.592	3,800- 31,350	3.64-5.00	0.700	15,200- 17,400	4.37-3.70				12	26+
-										12	25+
11+	None	5,800- 14,000	3.70-4.52							6	18+
9+	11.857	14,900- 56,100	4.75-5.40							6	17+
9+	19.737	14,000- 43,300	4.65-3.03 T-9							20	31+
4+	None	7,300- 600	3.42-1.81 T-21							14	19+
3+	None									24	30+
4½+	None	2,400- 10,000	4.10-4.60							2	7+
-										10	15+
4+	1.616	1,700- 10,450	1.38-2.64 T-4							18	22+
1½+										30	33+

[c]Figure represents the total number of millicuries administered, but leucocyte count never returned to approximate normal.

[d]Given x-ray therapy (300 r. units) terminally.

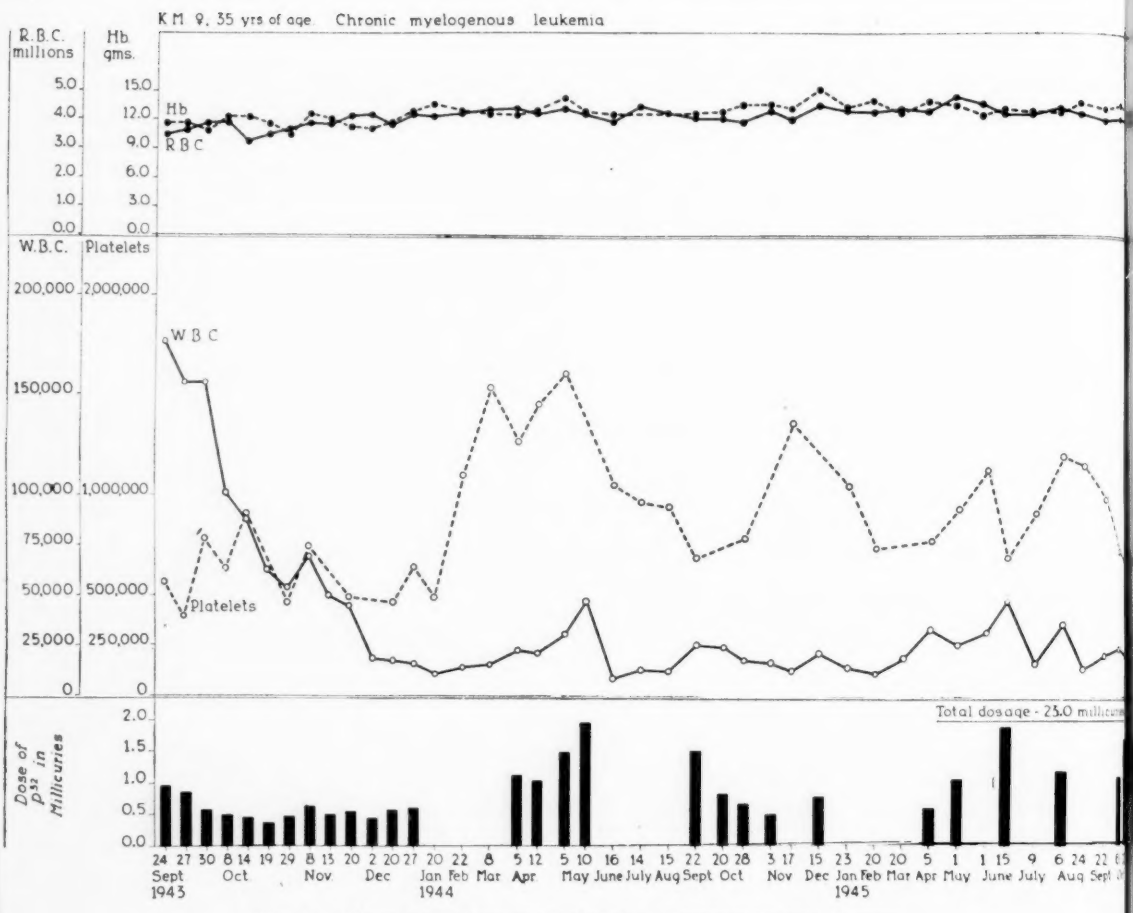
K M 9, 35 yrs of age Chronic myelogenous leukemia
R.B.C. millions
Hb gms.
Hb
R B C
W.B.C.
Platelets
W B C
Platelets
Total dosage - 23.0 millicuries
Dose of P32 in Millicuries
24 27 30 8 14 19 29 8 13 20 2 20 27 20 22 8 5 12 5 10 16 14 15 22 20 28 3 17 15 23 20 20 5 1 1 15 9 6 24 22
Sept Oct Nov Dec Jan Feb Mar Apr May June July Aug Sept Oct Nov Dec Jan Feb Mar Apr May June July Aug Sept
1943 1944 1945

Fig. 6.—Chronic myelogenous leukemia. Response to P^{32} therapy.

Case Summary.—K. M., a housewife, 35 years of age, began to notice large spontaneous bruises scattered over the body in June, 1943. Shortly thereafter she noted mild fatigability. These symptoms persisted, but there were no other complaints. She had had severe rheumatic fever at the age of 12 and a heart murmur ever since that time.

Physical Examination: Patient was moderately obese. There were four bruises on the legs and three on the arms. There was one small hemorrhage in the left fundus. The heart was moderately enlarged, and a loud, harsh, systolic murmur was heard at the apex. The splenic edge measured 6 cm. below the costal margin in the mid-clavicular line. The liver was not enlarged. There were no palpable lymph nodes.

Laboratory Data: Blood counts (Sept. 24, 1943): leucocytes, 175,000; erythrocytes, 3,480,000; hemoglobin, 11.5 Gm.; reticulocytes, 1.0 per cent; platelets, 557,000; differential: segmented polymorphonuclear neutrophiles, 43 per cent; stabs, 10 per cent; basophiles, 2 per cent; juveniles, 27 per cent; "C" myelocytes, 14 per cent; lymphocytes, 2 per cent; monocytes, 2 per cent. The sternal bone marrow on Sept. 24, 1943, was markedly hyperplastic, and the differential was: polymorphonuclear neutrophiles, 23 per cent; stabs, 25 per cent; juveniles, 19 per cent; "C" myelocytes, 22 per cent; "B" myelocytes, 6 per cent; "A" myelocytes, 2 per cent; eosinophiles, 1 per cent; primitives, 1 per cent; plasma cells, 1 per cent. There were 7 nucleated red blood cells per 100 myeloid cells. The urine was negative. The Kahn reaction was negative.

(Continued on opposite page.)

restored to more normal levels; in every case this second marrow examination showed fewer myeloblasts and myelocytes than did the original.

Table 6 shows not only the millicuries of P^{32} required to restore the leucocyte count to approximately normal levels, but also the amount given each year or fraction of a year thereafter in an attempt to keep the blood in as normal a state as possible. Eleven patients have been followed for more than a year since the leucocyte count was originally restored to the range of 4,000 to 15,000 cells per cubic millimeter; in all but one of these patients the leucocyte count subsequently rose to above 20,000 cells per cubic millimeter and further therapy was therefore given. The millicuries of P^{32} administered during this first "post-remission" year varied between 1.5 and 18.8 millicuries. Four of these patients have been followed for two years following the initial hematologic remission and have required from 1.6 to 11.9 millicuries during the second year. Only two patients have been followed for three years since their initial remission.

Charts showing the hematologic response of two of our patients to P^{32} therapy are shown in Figs. 6 and 7. The first of these represents a fairly typical case of chronic myelogenous leukemia; the patient is still living and the disease has been under good control for two years. The dosage of P^{32} which this patient has received is also about average. The second patient was thought to be in an acute or terminal phase of his illness when treatment was begun. Although the remission induced was of brief duration, the chart is presented to emphasize that even in this stage of the disease therapy may be of value.

2. *Effect of Therapy on the Clinical Manifestations of Myelogenous Leukemia:* The effect of radioactive phosphorus on the symptoms of these thirty-nine patients with myelogenous leukemia is shown in Table 7. Every symptom was included in this table, even though many of them were probably caused by incidental disorders other than the leukemia. Weakness, by far the most common symptom, was completely or partially relieved in 89 per cent of the patients. All symptoms except coughing and nervousness were completely relieved in at least 50 per cent of the patients. Of the four patients who complained of coughing, one was a heavy user of tobacco, and the other three were in the terminal phase of the disease when P^{32} therapy was started. Three of the nine patients who complained of nervousness had had this symptom virtually all their lives.

In Table 8 is summarized the effect of therapy on the abnormal physical signs. Splenomegaly is the only abnormal physical sign which did not disappear completely in at least 50 per cent of patients. The spleen was originally palpable in thirty-six of these patients; it became no longer palpable in ten patients (27.5 per cent), and definitely decreased in size but remained palpable in twenty-three patients (64 per cent). In only one instance where the patient lived longer than

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Course: The patient has had practically no symptoms since the onset of therapy with P^{32}. Within six weeks the spleen became no longer palpable; only on rare occasions since then has the tip of the spleen been felt on deep inspiration. She has had intermittent episodes of fatigue and nervousness, but her husband was in the Army, and she had four children to care for in addition to working regularly as a telephone switchboard operator. She is still alive and well (October, 1945).

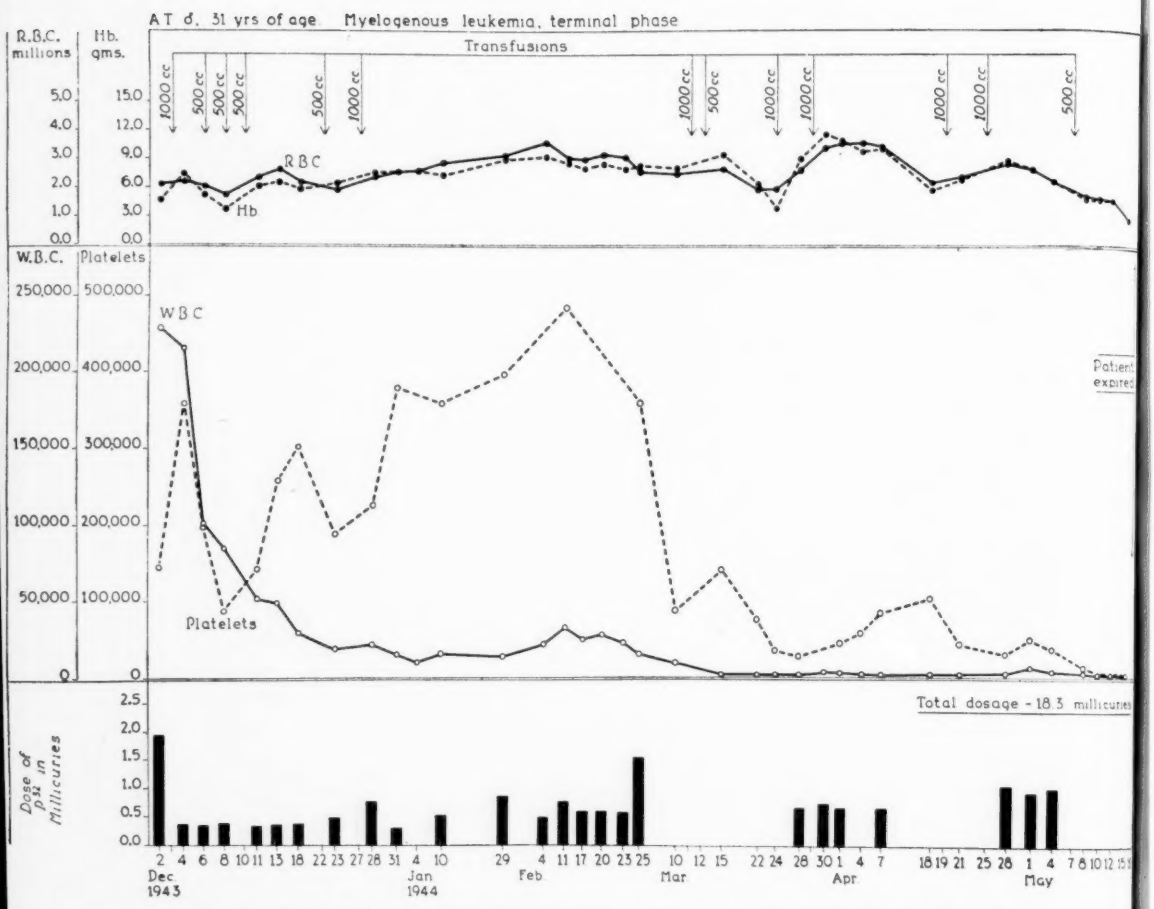


Fig. 7.—Terminal phase of myelogenous leukemia. Effect of P^{32} therapy on the blood.

Case Summary.—A. T., a man 31 years of age, began to have pain in both thighs and groins early in February, 1943. Shortly thereafter he had three shaking chills. The leucocyte count was found to be 266,000. Between February 23 and March 8 he was given x-ray therapy totaling 5,040 roentgen units. His symptoms improved and by June the white blood cell level had dropped to 5,800. He still had frequent headaches, cough, and persistent fever. In August, progressive anorexia occurred. The leucocyte level gradually rose, and, by September, 1943, reached 80,000. He was again given x-ray therapy, this time totaling 2,240 roentgen units. The white blood cell level decreased, but symptoms did not improve. During November, 1943, he developed progressive anorexia, severe pain in the chest, palpitation, dyspnea, orthopnea, increasing fever, and night sweats. Several days before he came to St. Louis for the first time he became mentally confused, and, at times, delirious. He was admitted to Barnes Hospital Dec. 2, 1943.

Physical Examination (Dec. 4, 1943): The temperature was 39.2° C. and the patient was semistuporous. There were several hemorrhages in the left fundus. The spleen measured 14 cm., and the liver 8 cm. below the left and right costal margins respectively (in midclavicular line). The lymph nodes were not enlarged. There was slight pitting edema of the feet.

(Continued on opposite page.)

TABLE 7. THIRTY-NINE PATIENTS WITH MYELOGENOUS LEUKEMIA—SYMPTOMATIC RESPONSE TO P^{32} THERAPY

SYMPTOM	NUMBER OF PATIENTS WHO HAD SYMPTOM AT ONSET OF THERAPY	% OF PATIENTS COMPLETELY RELIEVED OF SYMPTOM	% OF PATIENTS PARTIALLY RELIEVED OF SYMPTOM	% OF PATIENTS UNIMPROVED OR WORSE	% OF PATIENTS WHO LIVED LESS THAN 5 DAYS
Weakness and fatigability	36	55.5	33.5	5.5	5.5
Pounding in head	1	100	0	0	0
Dizziness	6	50	16.5	33.5	0
Dyspnea on exertion	16	50	25	18.5	6.5
Palpitation	8	62.5	25	12.5	0
Headaches	3	100	0	0	0
Tinnitus	1	100	0	0	0
Spontaneous bruising	4	50	25	25	0
Bleeding from nose or gums, or vagina (excessive)	9	89	0	11	0
Diarrhea	2	100	0	0	0
Bloating, dyspepsia	19	58	26.5	10.5	5
Nausea and/or vomiting	8	75	0	25	0
Anorexia	12	58.5	16.5	8.5	16.5
Abdominal pain	10	60	20	10	10
Cough	4	0	0	100	0
Blurring of vision	2	100	0	0	0
Pain in extremities	7	100	0	0	0
Soreness of gums	1	100	0	0	0
Semiconsciousness or stupor	1	100	0	0	0
Night sweats	1	100	0	0	0
Chills and feverishness	5	60	20	0	20
Nervousness	9	22	44.5	33.5	0
Swelling of legs or genitals	8	75	12.5	12.5	12.5

Laboratory Data: Blood counts (Dec. 2, 1943): leucocytes, 227,000; erythrocytes, 2,070,000; hemoglobin, 4.7 Gm.; reticulocytes, 2.0 per cent; platelets, 1,450,000; differential: segmented polymorphonuclear neutrophiles, 34 per cent; stabs, 4 per cent; juveniles, 7 per cent; myelocytes, 36 per cent; myeloblasts, 13 per cent; monocytes, 1 per cent. Sternal bone marrow examination was not done on admission (patient too ill). Urine was negative except for a trace of albumin. Kahn reaction was negative.

Course: Sulfamerazine therapy was started December 4 and for the next month the blood level was maintained between 5 and 10 mg. per cent. During the first few days in the hospital he had repeated epistaxes. On the sixth day of therapy with P^{32} he began to feel stronger and from then on showed progressive symptomatic improvement. As the leucocytic level decreased, the differential improved, and when he was discharged from the hospital thirty-nine days after admission, there were only 3 per cent myelocytes and no myeloblasts in the blood. During the next two and one-half months he continued to feel quite well except for moderate fatigability. He continued to run a low-grade fever. On March 11 and 12 he had several blood transfusions accompanied by severe reactions; thereafter the temperature rose too 39° C. every afternoon. He became gradually weaker, lips and gums became sore, and he had frequent epistaxes. During April, 1944, he became jaundiced. The gums began to bleed and numerous petechiae appeared. He died five and one-half months after the initial P^{32} therapy.

At post-mortem examination there were numerous petechiae in the skin, mucous membranes, and serous surfaces; the spleen weighed 1,600 grams, the liver 2,800 grams, and there was generalized hyperplasia of the lymph nodes. There was a focal atelectasis and congestion of all lobes of the lungs. Sections of sternal, rib, vertebral, and femoral marrows showed a slight increase of the myeloid elements of the marrow; a few foci of erythropoiesis and a few megakaryocytes were seen.

TABLE 8. THIRTY-NINE PATIENTS WITH MYELOGENOUS LEUKEMIA—CHANGES IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY

SYMPTOM	NUMBER OF PATIENTS WHO HAD ABNORMAL PHYSICAL SIGN AT ONSET OF THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING COMPLETELY DISAPPEARED AFTER THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING PARTIALLY SUBSIDED	% OF PATIENTS IN WHOM THERE WAS NO IMPROVEMENT	% OF PATIENTS IN WHOM DEATH OCCURRED IN LESS THAN 5 DAYS
Fever	14	64.5	7	21.5	7
Tachycardia	19	58	21	16	5
Petechiae or ecchymoses: skin	7	71	0	29	0
Petechiae or ecchymoses: mucous membranes	3	100	0	0	0
Bleeding from nose or gums	2	100	0	0	0
Enlarged lymph nodes	26	50	23	23	4
Splenomegaly	36	27.5	64	3	5.5
Hepatomegaly	29	51.5	27.5	14	7
Systolic cardiac murmur	19	63	16	10.5	10.5
Edema of extremities	6	50	33	17	0
Ascites	2	100	0	0	0

five days after therapy was started did the spleen fail to decrease in size, at least temporarily. Twenty-six patients had palpably enlarged lymph nodes, but in twelve of these the enlargement was minimal (largest nodes, from 0.5 to 1 cm. in diameter). The lymphadenopathy disappeared entirely in thirteen patients (50 per cent of those having this abnormality), and the lymph nodes decreased in size in six (23 per cent).

The results are recorded for each patient in Appendix Tables 2 and 3. Only the initial response to therapy is tabulated. As the disease progressed, both symptoms and physical signs which had at first improved or disappeared tended to recur. For example, practically all of the patients who have died experienced extreme weakness just prior to death. In a few instances an enlarged spleen which decreased greatly during the early stages of treatment subsequently enlarged again until the organ was bigger than it had been at the time treatment was first started.

Two of the patients in this series had positive Kahn reactions which were almost certainly "false positives." One of these two was a housewife, 37 years of age, who had been married for nineteen years. She had no history of primary or secondary syphilitic lesions, and no signs or symptoms of cardiovascular or neurosyphilis. Blood obtained from her husband and son gave negative Kahn reactions. Her leukemia responded well to therapy, and following restoration of the blood cytology to approximately normal levels, her Kahn reaction became negative. The other patient was a minister, 76 years of age. Again there was no history nor any physical signs suggestive of syphilis. This patient responded very poorly to treatment. His total leucocyte count dropped to approximately normal levels, but the profound anemia persisted in spite of repeated blood transfusions. The Kahn test was repeated five months after treatment was started and was again positive.

One patient had tremendously enlarged lymph nodes, liver, and spleen which failed to decrease in size following P^{32} administration but diminished

markedly following a subsequent course of x-ray therapy. At the onset of treatment, this patient, a 26-year-old man, had no complaints except weakness, and progressive enlargement of nodes in the cervical, axillary, epitrochlear, and inguinal regions. Some of these nodes were from 2 to 3 cm. in diameter. The spleen measured 8 cm. and the liver 9 cm. below the left and right costal margins, respectively, in the mid-clavicular lines. The leucocyte count was 26,000 per cubic millimeter, the differential showed 12 per cent juveniles and 10 per cent myelocytes, the erythrocyte level was 5,170,000, and platelets 486,000 per cubic millimeter. He was given 5.94 millicuries of radioactive phosphorus during a period of thirty-four days. At the end of this time the leucocyte count was 7,800 per cubic millimeter with only 6 per cent juveniles and 1 per cent myelocytes, the erythrocyte level was down to 3,750,000, and the platelet level remained normal. In spite of this lowering of the white blood cell level, the axillary and femoral nodes had increased about 50 per cent in size, the spleen now measured 9 cm., and the liver 9.5 cm. below the costal margins. No further therapy was given during the next two weeks, and the axillary nodes enlarged slightly. The patient developed dyspnea and coughing; fluoroscopic examination revealed enlargement of upper mediastinal lymph nodes. X-ray therapy was then started; during the next three weeks the patient received 600 roentgen units (2 doses of 300 roentgen units) to each of the following ports: right neck, left neck, anterior mediastinum, posterior mediastinum, left upper quadrant, right upper quadrant, left groin, and right groin. During this course of treatment, the cervical lymph nodes decreased considerably in size, and the inguinal nodes decreased slightly. The liver and spleen did not diminish in size. Following x-ray treatment, the leucocyte level ranged between 1,900 and 4,400 cells per cubic millimeter, the erythrocyte count dropped to 1,195,000, and platelets virtually disappeared from the blood. The patient developed enormous anasarca and died twelve days after the last x-ray treatment. In Fig. 8 is illustrated the response of the enlarged cervical nodes to x-ray therapy after P^{32} had failed to produce any improvement. This reduction in the size of the cervical nodes relieved the choking sensation temporarily, but there was no other evidence that the therapy was beneficial. Undoubtedly, the dosage of radiation delivered to the involved nodes was the determining factor in this response. This case is presented to emphasize that a mass of lymph nodes which is causing severe discomfort by virtue of its size can be reduced more readily by x-ray than by P^{32}. Thus, roentgen radiation can be used to supplement radioactive phosphorus therapy when a localized effect is needed.

C. *Comparison of Radioactive Phosphorus With Other Forms of Therapy Used in the Treatment of Myelogenous Leukemia.*—From the results obtained in the treatment of myelogenous leukemia, it appears that radioactive phosphorus (1) has not succeeded in curing any patients with the disease, (2) has very little effect on the course of patients with acute or subacute myelogenous leukemia, (3) produces clinical and hematologic remissions roughly comparable to those induced by x-radiation and Fowler's solution in nearly all subjects with the chronic form of the disease. Since the ability of all known forms of therapy to produce a cure or to influence favorably the acute form of myeloid leukemia can

TABLE 8. THIRTY-NINE PATIENTS WITH MYELOGENOUS LEUKEMIA—CHANGES IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY

SYMPTOM	NUMBER OF PATIENTS WHO HAD ABNORMAL PHYSICAL SIGN AT ONSET OF THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING COMPLETELY DISAPPEARED AFTER THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING PARTIALLY SUBSIDED	% OF PATIENTS IN WHOM THERE WAS NO IMPROVEMENT	% OF PATIENTS IN WHOM DEATH OCCURRED IN LESS THAN 5 DAYS
Fever	14	64.5	7	21.5	7
Tachycardia	19	58	21	16	5
Petechiae or ecchymoses: skin	7	71	0	29	0
Petechiae or ecchymoses: mucous membranes	3	100	0	0	0
Bleeding from nose or gums	2	100	0	0	0
Enlarged lymph nodes	26	50	23	23	4
Splenomegaly	36	27.5	64	3	5.5
Hepatomegaly	29	51.5	27.5	14	7
Systolic cardiac murmur	19	63	16	10.5	10.5
Edema of extremities	6	50	33	17	0
Ascites	2	100	0	0	0

five days after therapy was started did the spleen fail to decrease in size, at least temporarily. Twenty-six patients had palpably enlarged lymph nodes, but in twelve of these the enlargement was minimal (largest nodes, from 0.5 to 1 cm. in diameter). The lymphadenopathy disappeared entirely in thirteen patients (50 per cent of those having this abnormality), and the lymph nodes decreased in size in six (23 per cent).

The results are recorded for each patient in Appendix Tables 2 and 3. Only the initial response to therapy is tabulated. As the disease progressed, both symptoms and physical signs which had at first improved or disappeared tended to recur. For example, practically all of the patients who have died experienced extreme weakness just prior to death. In a few instances an enlarged spleen which decreased greatly during the early stages of treatment subsequently enlarged again until the organ was bigger than it had been at the time treatment was first started.

Two of the patients in this series had positive Kahn reactions which were almost certainly "false positives." One of these two was a housewife, 37 years of age, who had been married for nineteen years. She had no history of primary or secondary syphilitic lesions, and no signs or symptoms of cardiovascular or neurosyphilis. Blood obtained from her husband and son gave negative Kahn reactions. Her leukemia responded well to therapy, and following restoration of the blood cytology to approximately normal levels, her Kahn reaction became negative. The other patient was a minister, 76 years of age. Again there was no history nor any physical signs suggestive of syphilis. This patient responded very poorly to treatment. His total leucocyte count dropped to approximately normal levels, but the profound anemia persisted in spite of repeated blood transfusions. The Kahn test was repeated five months after treatment was started and was again positive.

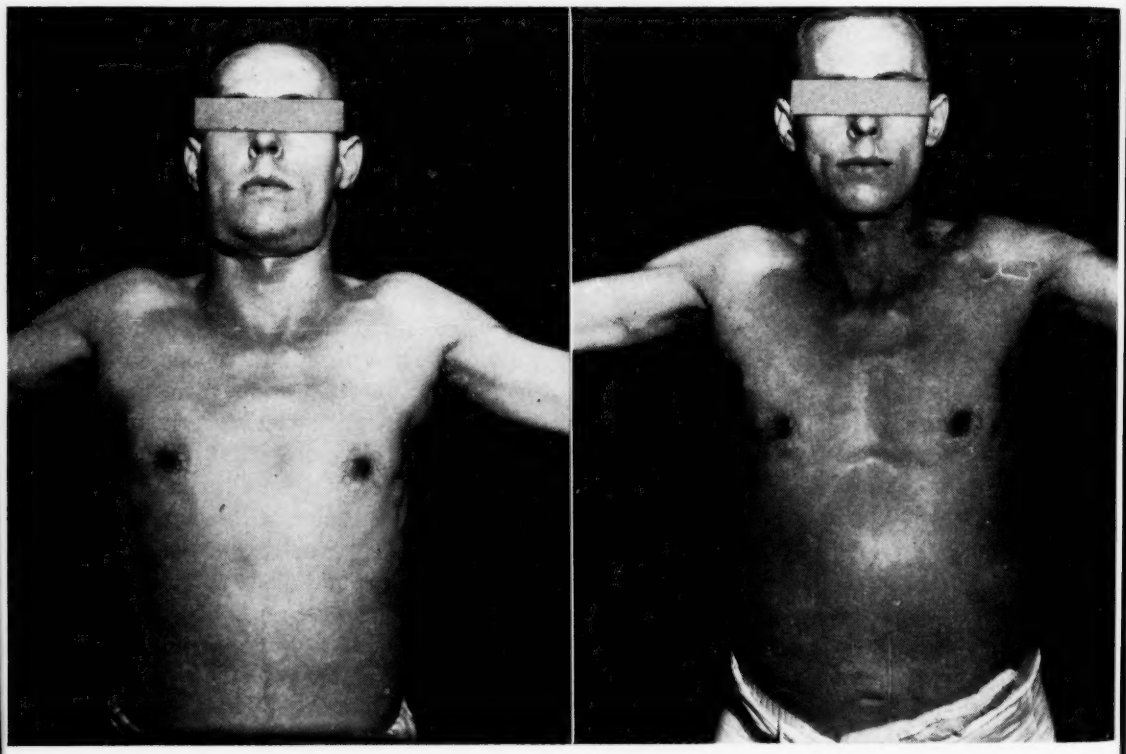
One patient had tremendously enlarged lymph nodes, liver, and spleen which failed to decrease in size following P^{32} administration but diminished

markedly following a subsequent course of x-ray therapy. At the onset of treatment, this patient, a 26-year-old man, had no complaints except weakness, and progressive enlargement of nodes in the cervical, axillary, epitrochlear, and inguinal regions. Some of these nodes were from 2 to 3 cm. in diameter. The spleen measured 8 cm. and the liver 9 cm. below the left and right costal margins, respectively, in the mid-clavicular lines. The leucocyte count was 26,000 per cubic millimeter, the differential showed 12 per cent juveniles and 10 per cent myelocytes, the erythrocyte level was 5,170,000, and platelets 486,000 per cubic millimeter. He was given 5.94 millicuries of radioactive phosphorus during a period of thirty-four days. At the end of this time the leucocyte count was 7,800 per cubic millimeter with only 6 per cent juveniles and 1 per cent myelocytes, the erythrocyte level was down to 3,750,000, and the platelet level remained normal. In spite of this lowering of the white blood cell level, the axillary and femoral nodes had increased about 50 per cent in size, the spleen now measured 9 cm., and the liver 9.5 cm. below the costal margins. No further therapy was given during the next two weeks, and the axillary nodes enlarged slightly. The patient developed dyspnea and coughing; fluoroscopic examination revealed enlargement of upper mediastinal lymph nodes. X-ray therapy was then started; during the next three weeks the patient received 600 roentgen units (2 doses of 300 roentgen units) to each of the following ports: right neck, left neck, anterior mediastinum, posterior mediastinum, left upper quadrant, right upper quadrant, left groin, and right groin. During this course of treatment, the cervical lymph nodes decreased considerably in size, and the inguinal nodes decreased slightly. The liver and spleen did not diminish in size. Following x-ray treatment, the leucocyte level ranged between 1,900 and 4,400 cells per cubic millimeter, the erythrocyte count dropped to 1,195,000, and platelets virtually disappeared from the blood. The patient developed enormous anasarca and died twelve days after the last x-ray treatment. In Fig. 8 is illustrated the response of the enlarged cervical nodes to x-ray therapy after P^{32} had failed to produce any improvement. This reduction in the size of the cervical nodes relieved the choking sensation temporarily, but there was no other evidence that the therapy was beneficial. Undoubtedly, the dosage of radiation delivered to the involved nodes was the determining factor in this response. This case is presented to emphasize that a mass of lymph nodes which is causing severe discomfort by virtue of its size can be reduced more readily by x-ray than by P^{32}. Thus, roentgen radiation can be used to supplement radioactive phosphorus therapy when a localized effect is needed.

C. *Comparison of Radioactive Phosphorus With Other Forms of Therapy Used in the Treatment of Myelogenous Leukemia.*—From the results obtained in the treatment of myelogenous leukemia, it appears that radioactive phosphorus (1) has not succeeded in curing any patients with the disease, (2) has very little effect on the course of patients with acute or subacute myelogenous leukemia, (3) produces clinical and hematologic remissions roughly comparable to those induced by x-radiation and Fowler's solution in nearly all subjects with the chronic form of the disease. Since the ability of all known forms of therapy to produce a cure or to influence favorably the acute form of myeloid leukemia can

be dismissed from further consideration, comparison of relative therapeutic value can be limited to (1) the undesirable reactions produced by each form of therapy, (2) the completeness of remissions induced, and (3) their effect in prolonging life.

The oral or parenteral administration of radioactive phosphorus does not produce any discomfort in patients and does not cause radiation sickness. Patients who have previously been treated with x-ray and have had radiation



A. *B.*

Fig. 8.—Response of adenopathy to x-ray therapy after P^{32} failed to produce any improvement. *A*, Lymph node enlargement in a patient with myelogenous leukemia following P^{32} therapy. *B*, Same patient following completion of a course of x-ray therapy.

illness are most appreciative of the fact that they can be treated without experiencing this reaction. The principal toxic effects to be avoided are those of leucopenia, thrombocytopenia, or aplastic anemia;[19] these are reactions which may also be produced by overdosage with x-radiation or Fowler's solution. There are no toxic manifestations comparable to those frequently produced by Fowler's solution: gastrointestinal disturbances, exfoliative dermatitis, or hepatic damage. We believe that in the treatment of myelogenous leukemia, as in polycythemia vera, radioactive phosphorus is the most pleasant form of therapy for the patient.

Clinical remissions induced by radioactive phosphorus are roughly comparable to those obtained with other forms of therapy. Symptoms are often completely relieved and most of the abnormal physical signs disappear. Several patients (Table 6, Patients 2, 15, and 31) have had remissions of a year or more during which their blood showed practically no leukemic changes, their symptoms were minimal, and no therapy was given. While it is rare to obtain similar results with Fowler's solution unless a maintenance dose of the drug is regularly given, comparable remissions are not unusual after x-ray treatment and may even occur spontaneously. In an occasional person, relief of joint or deep bone pain, or reduction in the size of lymph nodes which may be causing pressure symptoms, may not be as complete with radioactive phosphorus as with x-ray.

More experience will be required before the conclusion may unequivocably be drawn that radioactive phosphorus does or does not prolong the life of patients with myelogenous leukemia. It is already obvious, however, that the course of the disease is not greatly lengthened. The average duration of the disease from onset of symptoms to start of therapy with P^{32} in the twenty-one patients of our series who already have died was 32.6 months; the average duration from onset of symptoms to death was 42.4 months or 3.64 years. The average length of life during which P^{32} was administered, therefore, was only ten months, but these figures are weighted against the effectiveness of the isotope because all patients are included and some lived for only three to ten days after the initial injection. Also, some had previously been treated with x-radiation and their sensitivity to irradiation may have been decreased. Similar figures for the eighteen subjects still living should also be given: duration of disease from initial symptoms to first treatment with P^{32}, 15.1 months; duration from initial symptoms to Sept. 1, 1945, 31.1 months. Three of these patients have now been treated for more than three years and are still feeling well. When these data are compared with those published in the literature, however, they are not particularly impressive. Wintrobe[48a, b] found that the average duration of life following the onset of symptoms in 259 patients with chronic myelocytic leukemia reported by various authors was 3.28 years. Nathanson and Welch[49] found the median life expectancy for such patients to be about forty months. Minot, Buckman, and Isaacs[50a] reported that the average duration of life after the first symptom in fifty-two nonirradiated patients with chronic myelogenous leukemia was 3.04 years and in sixty-eight irradiated patients, 3.5 years. Individuals with the disease not infrequently live for more than five years. It appears, therefore, that radioactive phosphorus as it is used now does not greatly prolong the life of patients with chronic myelogenous leukemia; if it is any more effective than x-ray in this regard, the difference is measurable in terms of a few months and not in years.

D. *Summary and Recommended Method for Administering P^{32} to Patients With Chronic Myelogenous Leukemia.*—Our results corroborate the experience of previous investigators that in chronic myelogenous leukemia radioactive phosphorus therapy is effective in producing hematologic remission. The improvement in the blood and bone marrow cytology is accompanied in most cases by

striking relief of symptoms and marked improvement in the abnormal physical signs. The treatment does not produce radiation sickness, or any other subjective disturbances. The data on our patients suggest that radiophosphorus therapy is comparable to x-radiation with respect to prolongation of life.

In view of the extreme variation in the dosage of radioactive phosphorus required to bring about a restoration of the blood cytology to normal, no fixed schedule of treatment can be employed. However, the following routine has been found satisfactory for the first two weeks of therapy:

DAY OF TREATMENT	DOSAGE IN MC. (INTRAVENOUS)
1	1-2
3	0.5-1
6	0.5-1
10	0.5-1
14	0.5-1

The weight of the patient, the extent of the leucocytosis, and the degree of bone marrow hyperplasia are the determining factors in deciding whether to employ the smaller or the larger dosage. After the second week, and at times even before then, treatment must be individualized, depending upon the initial response. In patients having a very high initial leucocyte level, a safe procedure is to continue to administer from 0.5 to 1.0 millicuries at weekly intervals until the white blood cell level drops to approximately 30,000 cells per cubic millimeters. Further administration is then either withheld, or the dosage is decreased and the interval between injections increased. If the radiophosphorus be given orally, the above-mentioned doses should be multiplied by 4/3.

It is not implied that these recommendations represent the optimum treatment schedule. The ideal dosage per injection and the most satisfactory interval between injections are unknown. This tentative schedule merely represents one fairly satisfactory method of administering radiophosphorus. Actually, there was relatively little difference in the clinical or hematologic response of those patients who received small doses at frequent intervals and those who received larger doses at less frequent intervals. Furthermore, one usually can safely condense the course of treatment so that the leucocytes may be lowered to near-normal levels within two to three weeks rather than within the one to two months required with the more conservative schedule outlined. There is probably more danger of depressing erythropoiesis and platelet formation, however, when dosage is concentrated.

Several investigators have advocated that the amount of radioactive phosphorus to be given should always be calculated on the basis of millicuries per kilogram of body weight. In other words, an individual weighing 100 kilograms should receive exactly twice as great an initial dosage as would be given to a person weighing 50 kilograms. Although this is a theoretically sound conception, in actual practice body weight is of little value in determining the dosage for a given patient because of variation in susceptibility of leukemic cells to irradiation. In the last analysis, the optimum total dosage for any patient will depend on the observed response of that patient to therapy.

V. LYMPHATIC LEUKEMIA

The classification of the various types of lymphatic leukemia is a controversial subject. This is particularly true of that relatively large group of acute leukemias which many hematologists classify as leucosarcoma. Recognition of this particular type of acute leukemia is by no means universal. The distinction has to do with pathogenesis. If the disease is more closely related to lymphosarcoma than to chronic lymphatic leukemia, then its designation as leucosarcoma would seem to be preferable, whereas if the reverse is true, then acute lymphatic leukemia would seem more logical. As the etiology of all forms of leukemia is unknown, this controversy is of little practical importance. We have chosen, however, to use the terms leucosarcoma and acute lymphatic leukemia as defined by Wiseman[51] in classifying our own patients. The distinction between leucosarcoma and subacute or acute forms of lymphatic leukemia is made primarily on the basis of the morphologic appearance of the cells in supravitally stained preparations.[51]

In order to avoid confusion, both in discussing the literature and in analyzing our own cases, we shall consider all types of lymphatic leukemia under two headings: 1. *Chronic and Subacute Lymphatic Leukemia* and 2. *All Acute Types of Lymphatic Leukemia Including Leucosarcoma.*

A. *Review of the Literature.*—There are ten reports in the literature dealing with the therapeutic effectiveness of radioactive phosphorus for lymphatic leukemia.[1-3, 5, 7, 8-10, 12, 15] They are based on the study of 120 patients of whom sixty-four had chronic or subacute lymphatic leukemia and fifty-six had some form of acute lymphatic leukemia (including leucosarcoma).

The first case was reported in Lawrence's original paper.[1] The patient was a girl, 24 years of age, who first noticed enlarged cervical lymph nodes in February, 1937. In May, 1937, the diagnosis of lymphatic leukemia was made, and she was given x-ray therapy. Further x-ray was given in August, and again in October of the same year. Following each course of therapy, the leucocyte level fell (to as low as 600 cells per cubic millimeter), only to rise again rapidly. Toward the end of November she developed ulcerations of the gums, a leukemic skin rash, fever, and pain in several joints. By Dec. 14, 1937, the white blood cell level had risen to 116,800 cells per cubic millimeter, of which 97 per cent were lymphocytes; the hemoglobin was about 50 per cent. Between Dec. 14, 1937, and Feb. 24, 1938, she was given orally seventeen doses of P^{32} totaling 44.1 millicuries. The leucocyte level first decreased to 8,300 cells per cubic millimeter, but later rose to 93,000 in spite of continued treatment. Her general condition showed a progressive decline. Bleeding of gums and diarrhea occurred; she became comatose and died two days after taking the last dose of P^{32}.

Since this original report, specific data have been published for thirty-one cases of chronic or subacute lymphatic leukemia and for nineteen cases of the acute forms of the disease. Twenty-five of these chronic or subacute cases and sixteen of the acute ones were included in the comprehensive report of Erf, Tuttle, and Lawrence.[5] A summary of the symptoms and physical signs before and after treatment was given for all of these patients, but hematologic data were summarized for only two of the acute and eight of the chronic cases. Of the twenty-five patients with chronic lymphatic leukemia, eight had partial and one had a complete remission; of the sixteen patients with acute lymphatic leukemia, only one had a partial remission and one had a complete remission. At the time of the report, thirteen of the patients with the chronic form of the disease and all but one of those with the acute form were dead. The authors concluded radiophosphorus is as effective in relieving the clinical manifestations as are other types of treatment in general use. In all eight of the patients with chronic

TABLE 9. SUMMARY OF REPORTS FROM THE LITERATURE ON PATIENTS WITH LYMPHATIC LEUKEMIA TREATED WITH P^{32}

AUTHOR AND REFERENCE	PATIENT	AGE AND SEX	APPROX. INITIAL W.B.C. COUNT (PER C.MM.)	TREATMENT	COMMENTS
Warren[2]	1	55, M	20,500	5 injections of P^{32} totalling 9.1 mc. during period of 5½ months	*Acute lymphatic leukemia.* When patient was first seen he had pneumonia, for which x-ray therapy was given to the left chest; following this, leucocyte level dropped from 118,500 to 20,500 on day P^{32} therapy was started; 13 days later W.B.C. count was 7,300; it remained normal throughout the 6 months he was followed; clinical condition remained "fair"
	2	68, M	42,550	5 injections of P^{32} totalling 13.1 mc. during period of 5 months	*Acute lymphatic leukemia.* Within 6 weeks leucocyte level dropped to 11,600; it remained at about this level throughout remaining 3½ months of observation; R.B.C. level rose but patient received 3 transfusions; when last seen, he was still working steady
	3	57, M	126,500	4.0 mc. given I.V. June 28; 1 mc. I.V. July 11	*Subacute lymphatic leukemia.* Total leucocyte count dropped to 77,750 on 11th day after treatment was started; differential continued to show 100% lymphocytes and bone marrow showed 96% lymphocytes
Kenney and associates[8]	1	38, F	6,600 (89% lymphoid cells in the bone marrow)	12.3 mc. given in 11 doses from 4/25/41 to 5/16/41	*Leucosarcoma.* Roentgen therapy given prior to P^{32} was ineffectual; P^{32} was likewise of no benefit, and patient died 1 month after this treatment was started
Kenney[9]	1	Not given	20,000	16.1 mc. in 12 treatments during period of 120 days	*Chronic lymphatic leukemia.* Patient had just finished full course of x-ray therapy but without complete control of his disease; leucocyte level dropped to around 10,000, where it remained, and per cent of lymphocytes decreased from around 85 to about 65%
	2	58, M	27,200	9.1 mc. in 7 doses (from 11/19/40 to 1/20/41); 11.2 mc. in 6 doses (from 3/4/41 to 4/16/41)	*Chronic lymphatic leukemia.* Following P^{32} therapy there was virtually no change in either leucocyte count or per cent of lymphocytes; enlarged lymph nodes and spleen did regress in size, and symptomatically patient improved markedly
Low-Beer and associates[12]	1	41, M	1,020,000	12.05 mc. in 19 doses (from 10/25/41 to 1/16/42); 6.48 mc. in 8 doses (from 3/18/42 to 8/15/42)	*Chronic lymphatic leukemia.* Within 1 month after start of P^{32} spleen and lymph nodes had markedly decreased in size, and there was pronounced clinical improvement; on 1/30/42 W.B.C. count was 9,900 and R.B.C. level had increased from 2,960,000 to 5,790,000; when last seen (Aug., 1942), patient was working regularly as watchman

TABLE 9—CONT'D

AUTHOR AND REFERENCE	PATIENT	AGE AND SEX	APPROX. INITIAL W.B.C. COUNT (PER C.MM.)	TREATMENT	COMMENTS
Low-Beer and associates[12]	2	60, F	92,400	Not given; stated only that radiation level was kept at 3,000 mc. for 2 months; 1 month later patient was given 2.1 mc., 2 months later, 0.48 mc.	*Chronic lymphatic leukemia.* Definite improvement in blood condition was observed within 12 days (counts not given); lymph nodes and spleen decreased in size; patient regained her original weight
Warren[15]	1	49, M	296,000	Received 18.80 mc. orally (7 doses) and 17.98 mc. I.V. (8 doses) between 6/6/42 and 8/19/45	*Chronic lymphatic leukemia.* Leucocyte level dropped but remained elevated; patient had practically complete relief of symptoms and showed marked regression of enlarged lymph nodes, liver, and spleen. Patient was able to work steady

lymphatic leukemia for which hematologic data were given, the total leucocyte level showed a progressive lowering toward normal, but the percentage of lymphocytes decreased by more than 10 per cent in only five instances. The leucocyte level in one of the two patients with acute lymphatic leukemia for whom hematologic data were given rose from 3,000 cells per cubic millimeter before treatment to 6,000 per cubic millimeter three months later; in the other patient the white blood cells fell progressively from an initial count of 40,000 to 1,000 cells nine weeks after the first treatment. In both of these cases the percentage of lymphocytes decreased and the relative number of granulocytes increased following therapy. Erf and his associates concluded, as they had already done for myelogenous leukemia, that there was a less favorable response to therapy with P^{32} among those patients who had had previous x-radiation.

Detailed data have also been published by Warren,[2, 15] Kenney and his associates,[8, 9] and by Low-Beer, Lawrence, and Stone[12] for six other patients with chronic lymphatic leukemia and three with acute lymphatic leukemia. Brief summaries of all these cases are given in Table 9.

Craver[7] treated eleven persons with chronic lymphatic leukemia and thirteen with acute lymphatic leukemia; no data were recorded for any of these patients. Most of the eleven patients with chronic lymphatic leukemia were well along in the course of their disease and had previously been given x-ray therapy. In only three, or possibly four, of the cases was there evidence that the disease was controlled by P^{32} therapy. The dosage for those patients who were treated for any considerable period of time varied from 0.15 to 0.35 millicuries per kilogram of body weight. Craver concluded that the favorable effects seen in chronic lymphatic leukemia were mainly the regression of enlarged lymph nodes and spleens; in general, the blood and bone marrow were not notably influenced. Of the thirteen patients with acute lymphatic leukemia, two were adults and eleven were children. No benefit from administration of P^{32} was observed in either of the adults or in any of the nine children who had died. Two children were still living at the time of the report. In one of these there had been a slight effect on the size of lymph nodes. The other child had been under treatment for four months and showed a remarkable remission. Details concerning the remission were not given, but at the end of the four-month period a relapse occurred.

Kenney[9] treated eight children with acute or subacute lymphatic leukemia (the number in each catagory was not stated, and in making our analysis we put them all in the acute group as all were children and all but one died within four weeks). No data were given for any of these patients, but analysis of the results of treatment led Kenney to conclude that: "Radioactive phosphorus therapy has to date been no more effective in the acute or subacute leukemia of childhood than has any other form of radiation therapy; i.e., had no value at all."

Eight adults with chronic lymphatic leukemia were also treated with P^{32} by Kenney.[9] A case history was given for one patient and hematologic data and therapy were presented in a chart for a second patient; both of these cases are summarized in Table 9. From his experience in the treatment of these eight patients, Kenney drew the following conclusions: (1) Radioactive phosphorus should be administered cautiously to patients with aplastic or infiltrated bone marrow; (2) it has not been effective in patients who have become radiation fast; (3) it reduced the enlarged lymph nodes in four patients and reduced the spleen in three patients; (4) the leucocyte count was decreased in one of eight patients; (5) the proportion of lymphocytes in the differential count was altered in one of eight patients; (6) symptomatically, five of the eight patients were improved; (7) there was no radiation sickness.

The effect of P^{32} on four patients with chronic lymphatic leukemia was analyzed by Fitz-Hugh and Hodes,[10] but again no data were given. Two of the four patients with chronic lymphatic leukemia obtained good remissions, and one was still in excellent condition a year later. One very ill patient died shortly after the first administration, and the last patient had only recently been started on treatment at the time of the report. None of the four patients with acute lymphatic leukemia was benefited by radioactive phosphorus therapy.

The last report was that of Warren,[15] who studied twelve cases of chronic and subacute lymphatic leukemia and twelve cases of the acute form of the disease. The only one for which data were given is summarized in Table 9. Five of eight patients with chronic lymphatic leukemia, one of four with subacute lymphatic leukemia, and three of twelve with acute lymphatic leukemia were "helped." It is interesting that Warren reported a better response to therapy with P^{32} in those patients who had previously received roentgen therapy than in those who had received no x-ray; seven of eleven in the former group were "helped," whereas only two of thirteen in the latter group were benefited.

In summary, there is agreement among most of these investigators that P^{32} is effective in the majority of cases of chronic lymphatic leukemia in (1) restoring the leucocyte count to near normal levels, (2) reducing the size of enlarged lymph nodes and spleen, and (3) causing temporary amelioration of symptoms. Only three reports present sufficient data to permit comparison of the relative effectiveness of this form of therapy in chronic lymphatic leukemia and in myelogenous leukemia. Erf and associates[5] observed five complete remissions among forty-six patients treated for chronic myelogenous leukemia, whereas only one complete remission occurred among twenty-five patients with chronic lymphatic leukemia. Furthermore, although a fall in the leucocyte level to near-normal values was fairly constant in both groups, a significant rise in the erythrocyte level occurred more frequently in the myeloid group. Craver,[7] after treating eleven patients with each type of chronic leukemia, concluded that a favorable effect of P^{32} on the blood cytology was more constant in the chronic myeloid group. On the other hand, Warren[15] reported that 63 per cent of his patients with chronic lymphatic leukemia were helped by treatment, but only 47 per cent of the patients with chronic myelogenous leukemia were benefited.

B. *Analysis of Results Obtained in Treating Forty-Five Patients With Lymphatic Leukemia.*—Thirty patients with chronic or subacute lymphatic leukemia and fifteen patients with leucosarcoma (or acute lymphatic leukemia) have been treated with radioactive phosphorus. Of the patients in the former group, sixteen have died and fourteen are still living; all fifteen patients in the latter group have died.

1. *Chronic and Subacute Lymphatic Leukemia:* The same laboratory tests were done on these patients as on the subjects with chronic myelogenous leukemia. All persons with chronic lymphatic leukemia seen in this clinic in the last three and one-half years, except those who could not return for frequent follow-up visits, were treated with P^{32} and included in this series. The average duration of symptoms at the onset of therapy with P^{32} was nineteen months;

four of the patients had had specific symptoms referable to their leukemia for three years or longer. Thus, on the average, the duration of symptoms was shorter for this group of patients than for the group having myelogenous leukemia.

Hematologic and therapeutic data are summarized in Table 10. Twenty-one of the patients were men and nine were women. Only ten had had any other therapy prior to P^{32}; in eight of these cases the previous treatment included roentgen radiation. In four instances, x-radiation had been administered within one to three months prior to the first injection of P^{32}.

The initial leucocyte count was above 15,000 cells per cubic millimeter in twenty-four patients, between 15,000 and 4,000 in five, and below 4,000 in one. Seventeen of the thirty patients had an initial anemia of less than 4,000,000 cells per cubic millimeter, and in fourteen instances platelets were less than 300,000 per cubic millimeter.

As with other blood dyscrasias, there was considerable variation in the dosage of radioactive phosphorus required to restore the total leucocyte count to approximately normal levels. Of the twenty-four patients whose total leucocyte level was originally above 15,000 cells per cubic millimeter, twenty showed a restoration to normal values. The total dosage required to restore the leucocyte count to approximately normal varied from 1.6 to 11.2 millicuries; fourteen of these twenty patients received less than 6 millicuries. Thus, the average dosage was somewhat smaller than in the group of persons with chronic myelogenous leukemia.

Twenty-four of the thirty patients showed a significant increase in the percentage of granular cells and a decrease in the percentage of lymphocytes in the blood following administration of P^{32}. Five who did not show this change had a poor clinical response to therapy; the sixth patient had a normal differential at the time P^{32} was first given (the diagnosis was based on the presence of a leucopenia and the characteristic bone marrow changes of chronic lymphatic leukemia).

The erythrocyte level increased by more than a million cells per cubic millimeter in only one of the twenty-three subjects who had received no transfusions. Six patients who were given transfusions showed a progressive fall in the erythrocyte level after treatment. The platelet count was less than 300,000 per cubic millimeter at the onset of P^{32} therapy in fourteen patients; in three instances the thrombocyte level rose above 400,000, but in no case did it rise as high as 500,000 per cubic millimeter after therapy. Thus, the improvement in the erythrocyte and platelet levels which occurred so frequently following treatment of myelogenous leukemia was rarely observed in individuals with lymphatic leukemia.

Ten patients have been followed for a year or longer since the leucocyte level was originally restored to normal. In no case was the remission sufficiently complete or prolonged so that the individual remained in remission for a year or more without further treatment. The dosage of P^{32} administered during this first "post-remission" year varied from 1.4 to 19.0 millicuries. Five of these

TABLE 10. HEMATOLOGIC DATA ON TWENTY-SEVEN

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	BLOOD COUNTS AT TIME P^{32} THERAPY WAS STARTED						P^{32} THERAPY REQUIRED TO RESTORE W.B.C. COUNT TO APPROX. NORMAL (4,000-15,000)		MOST NORMAL BLOOD COUNTS AFTER P^{32} THERAPY					
			W.B.C. (PER C.MM.)	R.B.C. (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% GRANULOCYTES	% LYMPHOCYTES	MC.	DAYS	W.B.C. (PER CMM.)	R.B.C. (PER C.MM.)[b]	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% GRANULOCYTES	% LYMPHOCYTES
1	52, M	X-R	98,500	4.41	12.8	620,000	10	89	11.2	34	8,900	4.17	12.5	401,000	72	27
2	63, F	X-R	63,500	4.88	15.4	600,000	13	86.5	4.06	54	8,100	4.46	13.0	775,000	20	75
3	35, M		71,800	5.17	16.3	899,000	12	85	6.55	36	7,550	4.88	15.0	504,000	60	32
4	67, F		4,450	2.05	6.6	90,000	39	61	3.02[d]	134	5,100	2.97 T-5	9.7	174,000	52	46
5	58, F		16,700	4.15	11.7	880,000	55	40	2.926	15	10,000	3.97	11.0	670,000	63	27
6	58, M		40,300	4.06	9.4	382,000	18	81	4.931	33	11,050	4.56	10.7	467,000	58	35
7	58, M		132,000	2.00	6.7	172,000	9	89	6.912	28	8,050	3.64 T-2	12.1	210,000	33	55
8	51, M		18,100	4.72	14.1	370,000	60	35	3.987	29	8,300	4.53	13.6	795,000	67	24
9	61, M		21,050	4.00	13.5	632,000	41	57	3.433	28	17,350	4.06	11.0	755,000	70	25
10	46, M	X-R	17,500	5.10	15.8	816,000	47	48	1.642	33	11,700	5.08	15.8	920,000	59	38
11	60, M		471,000	1.83	4.6	91,000	2	98	9.654[c]	68	24,200	2.67 T-8	7.8	150,000	3	96
12	70, M	X-R	12,150	3.84	12.7	415,000	30	63	7.023[d]	112	5,900	4.16	13.7	458,000	54	39
13	75, M		444,000	2.82	10.5	705,000	3	96	9.012	112	14,500	4.05	11.5	1,027,000	51	42
14	53, M		28,500	4.11	12.7	788,000	15	81	2.718	30	6,400	4.25	14.4	1,207,000	51	45
15	66, M		13,650	3.45	10.5	569,000	20	80	2.367	7	8,550	3.77	11.9	360,000	64	24
16	62, M	X-R, T	6,750	0.95	1.0	260,000	15	82	8.446[d]	36	8,400	1.35 T-2	3.8	153,000	18	79
17	47, M	X-R	11,300	4.25	12.9	391,000	31	63	7.412[d]	533	7,650	4.58	13.4	484,000	52	41
18	59, F	Fo	81,500	2.45	7.2	201,000	7	91	5.010	14	7,850	3.24	10.7	480,000	42	51
19	39, M		23,900	4.40	13.4	290,000	46	49	5.667	10	10,550	4.00	12.1	385,000	45	51
20	64, M		59,700	4.04	13.5	218,000	9	90	10.852[c]	127	18,350	4.23	13.0	370,000	11	86
21	48, F	X-R	37,000	3.70	12.9	244,000	5	95	8.87	159	10,500	4.26	13.1	--	19	80
22	53, F		310,000	3.30	10.9	152,000	0.5	99.5	9.364[c]	48	325,000	2.68 T-5	8.8	32,000	1	99
23	47, M		2,700	2.25	6.3	90,000	52	34	2.361	6	5,100	3.65 T-11	11.7	476,000	66	28
24	54, M		36,500	4.61	15.0	837,000	10	88	6.799	49	5,600	5.56	14.9	558,000	68	19
25	56, M		66,500	1.08	3.8	119,000	6.5	92.5	4.156	12	4,200	3.33 T-11	8.9	138,000	46	48
26	52, F		382,000	3.07	9.6	708,000	1	98	18.865[c]	107	354,000	3.25	9.2	868,000	1	98
27	55, M	X-R	150,000	2.80	9.2	232,000	4	96	4.131	31	8,550	3.48	10.1	306,000	72	23
28	59, F		55,500	3.97	11.6	553,000	14	85	3.570	35	8,450	4.15	12.0	521,000	34	64
29	55, F	T	195,000	1.62	5.8	140,000	3	97	5.135	22	8,100	3.11 T-5	11.3	480,000	52	38
30	50, M		142,000	2.04	5.8	32,000	2	98	4.231	22	4,400	2.08	5.8	64,000	24	75

[a] X-R = X-ray therapy; Fo = Fowler's solution; B.M. = Bone marrow extract; T = Transfusions.

[b] T = Transfusions. The figure indicates the number of transfusions given.

[c] Figure represents the total number of millicuries administered, but leucocyte count never returned to approximately normal (4,000 to 15,000).

[d] Total leucocyte count approximately normal (4,000 to 15,000) at start of therapy. Figure represents the total number of millicuries administered.

PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA

DURATION FROM RESTORATION OF NORMAL W.B.C. COUNT UNTIL DEATH OR 9/1/45 (MO.)	TREATMENT AND BLOOD COUNTS DURING FIRST YEAR OR FRACTION THEREOF FOLLOWING RESTORATION OF LEUCOCYTE COUNT TO APPROXIMATELY NORMAL (4,000-15,000)			TREATMENT AND BLOOD COUNTS DURING SECOND YEAR OR FRACTION THEREOF FOLLOWING INITIAL RESTORATION OF LEUCOCYTE COUNT TO APPROX. NORMAL			TREATMENT AND BLOOD COUNTS DURING THIRD YEAR FOLLOWING INITIAL RESTORATION OF LEUCOCYTE COUNT TO NORMAL			DURATION OF DISEASE (MO.) FROM FIRST SYMPTOM	
	MC. OF P^{32}	W.B.C. RANGE (PER C.MM.)	R.B.C. RANGE[b] (PER C.MM.)	MC. OF P^{32}	W.B.C. RANGE (PER C.MM.)	R.B.C. RANGE[b] (PER C.MM.)	MC. OF P^{32}	W.B.C. RANGE (PER C.MM.)	R.B.C. RANGE[b] (PER C.MM.)	TO START OF P^{32}	TO DEATH OR 9/1/45
12	3.80	4,800-106,000	.72-3.94 T-9							36	49
38+	12.73	13,400- 34,000	3.73-4.71	13.01	8,100-29,800	2.14-4.19	10.84	17,300-33,400	3.66-446	14	53+
34	12.16	7,250- 27,700	3.95-5.00	8.38	6,000-25,700	3.75-4.70	10.90	800-41,450	.90-3.82 T-18	12	47
5[e]	3.02	850- 5,100	.90-2.97 T-10							24	29
2½	4.86	2,050- 10,050	2.22-3.64 T-1							8	11
33+	6.77	11,050- 25,500	3.57-4.55	9.771	8,100-26,850	2.14-3.83	7.21	7,300-30,150	2.22-3.27[f]	3	37+
2¼	0	7,750- ?	2.73-3.15 T-3							8	11
33+	18.99	6,650- 43,350	2.95-4.53	10.60	4,300-67,000	2.38-4.37	1.358	3,650-8,000	3.77-4.56	7	41+
1	0	5,800- 7,700	2.99-4.06							18	20
21	14.33	11,450- 22,150	4.58-5.21	7.75	12,750-38,800	4.41-4.89				24	46
2[e]	9.65[f]	24,000-500,000	1.44-2.18 T-10							72	74
27	3.026	4,100- 13,900	3.21-4.16	0	5,150-17,600	3.44-3.80	0	11,500-17,000	3.01-3.42	12	35+
7	3.17	9,400- 21,900	3.41-4.00							5	12
23+[g]	8.18	3,500- 14,500	2.26-4.38							4	26+
23+[g]	1.414	2,250- 8,550	2.17-3.77							18	41+
1½[e]	8.446[f]	4,850- 30,450	.58-1.35 T-13							5	6
21+[e]	6.368[f]	6,800- 35,500	4.16-4.79	0	5,100-16,600	4.03-4.45				21	42+
7	18.63	2,300-296,000	.99-3.24 T-16							15	22
4	1.712	10,500- 2,000	4.39-1.70 T-5							12	16
4½	10.852	18,350- 51,000	3.02-4.23							12	16½
4	4.31	3,300- 44,500	1.99-4.26 T-2							36	45
1½	9.364	310,000-390,000	1.63-3.70 T-5							?	?
10+	10.866	1,700- 4,800	.82-4.20 T-11							21	31+
7+	0	3,900- 5,800	3.65-5.56							12+	21+
4+	1.641	1,100- 5,500	1.06-3.33 T-19							1	5+
5[e]	18.865	286,000-562,000	3.80-1.62 T-6							12	17
3+	0	8,550- 14,400	2.86-3.13							120	123+
3+	1.380	8,450- 13,400	3.62-3.94							1½	5+
2+	0	14,100- 4,300	2.61-1.50 T-6							8	11+
1+										18	19+

[e]Duration from first treatment until death (or 9/1/45 if still living).

[f]Treatment in millicuries during the first twelve months (or fraction thereof) of P^{32} therapy.

[g]Patient is still living and working regularly two years after first treatment, but he has not been seen by us since eight months after first treatment.

patients have been followed for two years following the initial hematologic remission, and all but one of these received further therapy (8.4 to 13.0 millicuries) during the second year. Only one person has been under observation for a third year; she received 10.8 millicuries during this period.

The effect of P^{32} on the symptomatology of chronic lymphatic leukemia is shown in Table 11 (see Appendix Table 4 for complete tabulation). When first seen in our clinic, a good many of these patients had no complaints other than weakness and fatigability and enlargement of lymph nodes. Weakness and fatigability were completely or partially relieved in 78 per cent of individuals having these complaints; 22 per cent showed no significant improvement. Approximately one-half of all the symptoms encountered were completely relieved in at least 50 per cent of the patients. Not a single patient experienced

TABLE 11. THIRTY PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA—SYMPTOMATIC RESPONSE TO P^{32} THERAPY

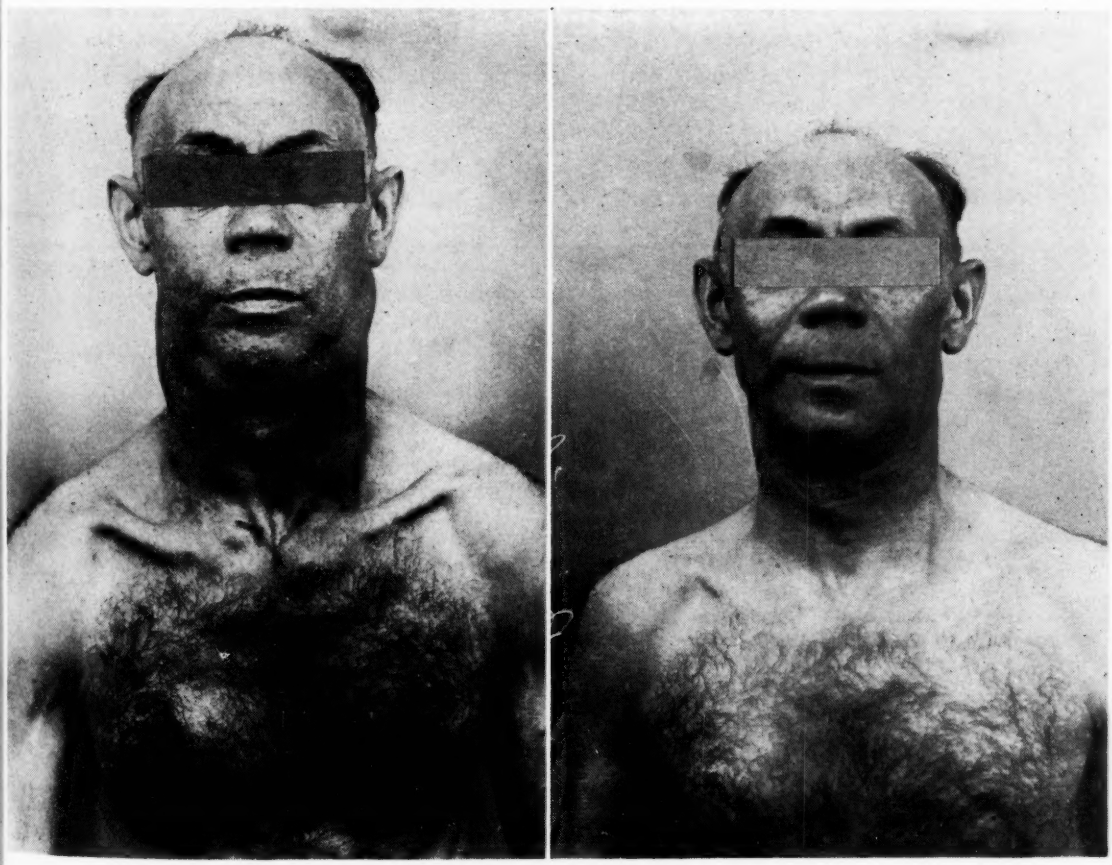
SYMPTOM	NUMBER OF PATIENTS WHO HAD SYMPTOM AT ONSET OF THERAPY	% OF PATIENTS COMPLETELY RELIEVED OF SYMPTOM	% OF PATIENTS PARTIALLY RELIEVED OF SYMPTOM	% OF PATIENTS UNIMPROVED OR WORSE
Weakness and fatigability	27	41	37	22
Pounding in head	1	0	0	100
Dizziness	6	33	0	67
Dyspnea on exertion	9	44.5	44.5	11
Palpitation	3	67	0	33
Headaches	4	50	0	50
Spontaneous bruising	3	67	0	33
Bleeding from nose or gums or vagina (excessive)	5	40	0	60
Bloating, dyspepsia	5	0	40	60
Nausea and/or vomiting	1	0	0	100
Anorexia	9	0	55.5	44.5
Abdominal pain	3	67	0	33
Cough	3	67	33	0
Pain in extremities	4	75	0	25
Soreness of gums	2	100	0	0
Excessive sweating	5	40	0	60
Swelling of legs or genitals	1	100	0	0

TABLE 12. THIRTY PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA—CHANGES IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY

SYMPTOM	NUMBER OF PATIENTS WHO HAD ABNORMAL PHYSICAL SIGN AT ONSET OF THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING COMPLETELY DISAPPEARED AFTER THERAPY	% OF PATIENTS IN WHOM ABNORMAL FINDING PARTIALLY SUBSIDED	% OF PATIENTS IN WHOM THERE WAS NO IMPROVEMENT
Fever	3	0	0	100
Tachycardia	7	0	14	86
Petechiae or ecchymoses — skin	8	62.5	0	37.5
Petechiae or ecchymoses — mucous membranes	4	75	0	25
Enlarged lymph nodes	29	10	55	35
Splenomegaly	24	21	58	21
Hepatomegaly	16	37.5	25	37.5
Systolic cardiac murmur	10	0	20	80
Edema of extremities	2	50	50	0
Ascites	1	0	0	100

complete relief of bloating, dyspepsia, or anorexia. In general, the symptomatic response to therapy was not as favorable as in the myelogenous leukemia group.

The effect of therapy on the abnormal physical signs is summarized in Table 12 (see Appendix Table 5 for complete tabulation). Hemorrhagic manifestations and edema of the extremities were the only two physical signs which disappeared entirely in as many as 50 per cent of the patients. An enlarged spleen became no longer palpable in only 21 per cent of the patients. Enlarged lymph nodes became no longer palpable in only 10 per cent, but in another 55 per cent there was some diminution in their size. In Fig. 9 are shown photographs of one of the patients before and after P^{32} therapy; the reduction in size of the cervical nodes following treatment is clearly visible. This patient was one of the 55 per cent in whom nodes diminished but never returned to normal size.



A. *B.*

Fig. 9.—Patient 1, a 52-year-old man, with chronic lymphatic leukemia. *A*, Cervical lymph node enlargement three days prior to therapy. *B*, Same patient two months after therapy with P^{32} had been started. Patient had received 11.6 millicuries (all given within the first thirty-four days).

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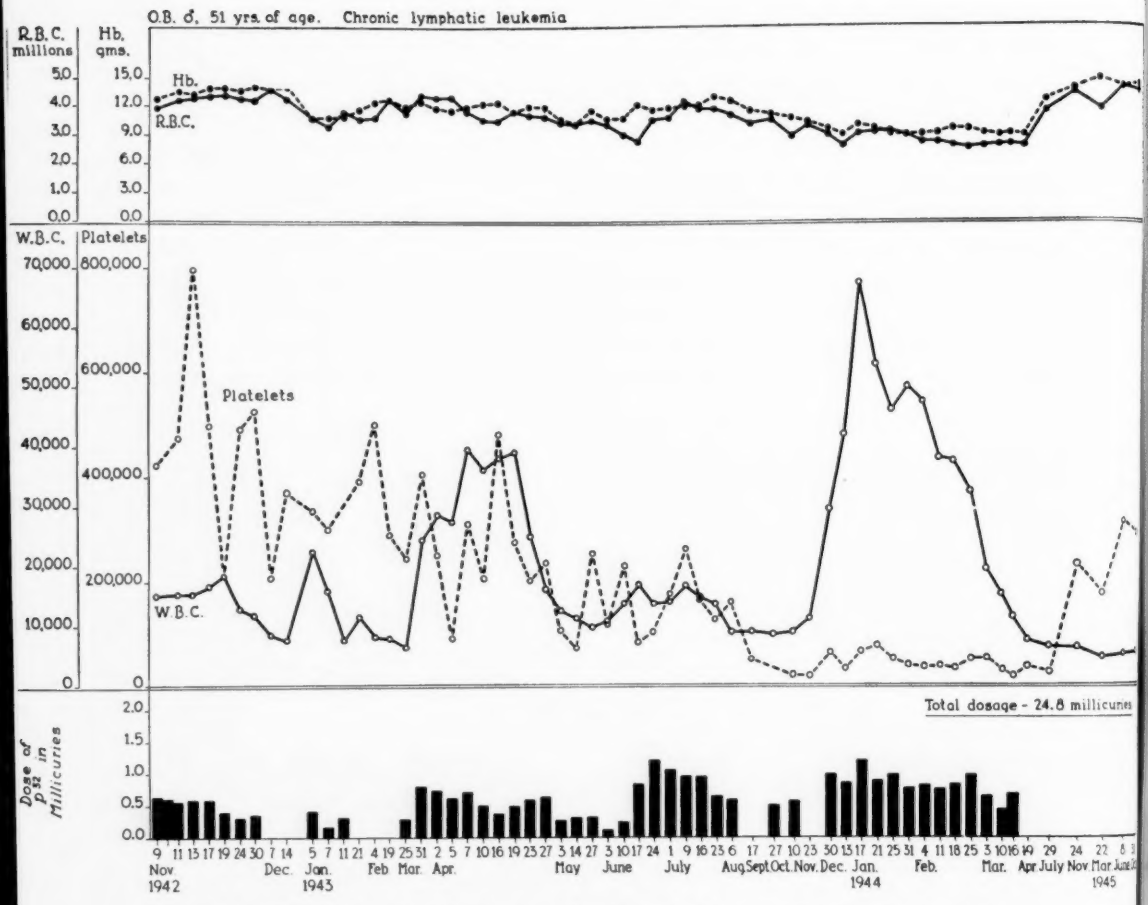

Fig. 10.—Chronic lymphatic leukemia. Hematologic changes following P^{32} therapy.

Case Summary.—O. B., a man 51 years of age, first noted enlarged nodes in the neck and right axilla in April, 1942. In October, 1942, there was a rapid increase in the lymph node enlargement. He became short of breath and fatigued easily. His legs began to ache.

Physical Examination (Nov. 5, 1942): There was striking enlargement of the lymph nodes in the cervical, axillary, and inguinal regions; one mass in the right axilla measured 5 cm. in diameter and numerous cervical nodes measured from 3 to 4 cm. in diameter. There was a subconjunctival hemorrhage (right eye). The spleen measured 7 cm. and the liver 4 cm. below the left and right costal margins, respectively (mid-clavicular line).

Laboratory Data: Blood counts (Nov. 9, 1942): leucocytes, 15,200; erythrocytes, 3,910,000; hemoglobin, 12.6 Gm.; reticulocytes, 2.8 per cent; platelets, 422,000; differential: polymorphonuclear segmented neutrophiles, 39 per cent; stabs, 8 per cent; eosinophiles, 2 per cent; basophiles, 1 per cent; monocytes, 6 per cent; lymphocytes, 44 per cent. Sternal bone marrow (Nov. 6, 1942): 91 per cent of the cells in the marrow were small lymphocytes, 3 per cent nucleated red blood cells. Urine, negative. Kahn reaction, negative.

Course: The patient felt progressively stronger and the lymph nodes decreased in size during the first six weeks of P^{32} therapy. He then developed tracheobronchitis and pneumonitis and on Dec. 31, 1942, the leucocyte count was 31,700, with 71 per cent lymphocytes. Following sulfamerazine therapy and further P^{32} he improved rapidly, and by the end of

(Continued on opposite page.)

In some patients the reduction in the size of the lymph nodes and spleen did not occur until many months after therapy with P^{32} was started. Patient 13 (Table 10) is a good example of this. Seven days after the onset of treatment, the lymph nodes showed little change in size; shortly after this they began to increase in size in spite of the fact that treatment was continued. On the fourteenth day of therapy (at which time the patient had received 4.1 millicuries of P^{32}), the lymph nodes in the neck and axilla were approximately 50 per cent larger than they had been initially. About a month later, following continued treatment, they began to recede, and two months later they were approximately one-half their original size.

In Patient 14 the cervical and axillary nodes were as large as 2 and 3 cm. in diameter, respectively. At first they decreased slightly in size, but later, even though administration of P^{32} was continued, the nodes again enlarged. Seven months after the beginning of therapy (total dosage, 10.9 millicuries), they were somewhat larger than they had been originally. At this time the leucocyte count had been normal for six months. The patient was then given, within a period of six days, 800 roentgen units of x-ray to each side of the neck and 400 roentgen units to each axilla. One month later the nodes had decreased about 50 per cent in size. It is now eighteen months since he was treated with the x-radiation. He has received no further therapy, is still working regularly, and is feeling fairly well. Unfortunately, we have not been able to persuade him to return to the clinic, and the present size of the nodes is unknown. Unquestionably, the lymph node enlargement responded more satisfactorily to x-radiation than to P^{32}.

Similarly, Patient 18 had tremendous splenomegaly which was totally unaffected by 13.1 millicuries of P^{32} given over a period of three months; the splenic measurement subsequently decreased from 25 cm. to 19 cm. below the costal margin following direct x-ray therapy to the spleen (two treatments of 400 roentgen units each). Bloating, belching, and anorexia remained prominent symptoms throughout the course of her illness.

The chief complaint of Patient 17 was soreness of the mouth and tongue. He had three firm, elevated, sharply demarcated plaques on the tongue; these

January, 1943, the blood cytology was within normal limits. He felt well until April, 1943, when the lymph nodes began to enlarge, and the spleen increased in size (measured 11 cm. below the costal margin). The leucocyte level rose to 43,300, with 78 per cent lymphocytes. At this time he felt very weak, had no appetite, and complained of pains in the legs and upper abdomen. Further P^{32} therapy was given, and again the symptoms subsided as the blood cytology returned to normal. He remained essentially asymptomatic until December, 1943, when he had "the flu." Weakness returned, gums became sore, lymph nodes enlarged, and numerous petechiae appeared on the trunk. By January 17, 1944, the leucocyte level was up to 67,000 (85 per cent lymphocytes) and the platelet count was 66,000. P^{32} therapy was intensified; the leucocyte returned to normal, but the platelet level dropped to between 50,000 and 15,000. He complained of pains in the back, legs, and left upper quadrant and had numerous petechiae and an occasional epistaxis. In April, 1944, he began to feel progressively stronger; all bleeding manifestations and other symptoms disappeared. He has remained essentially asymptomatic until the present (October, 1945). The lymph nodes on Oct. 31, 1945, were as large as 2 cm. in diameter; the spleen was felt just at the costal margin.

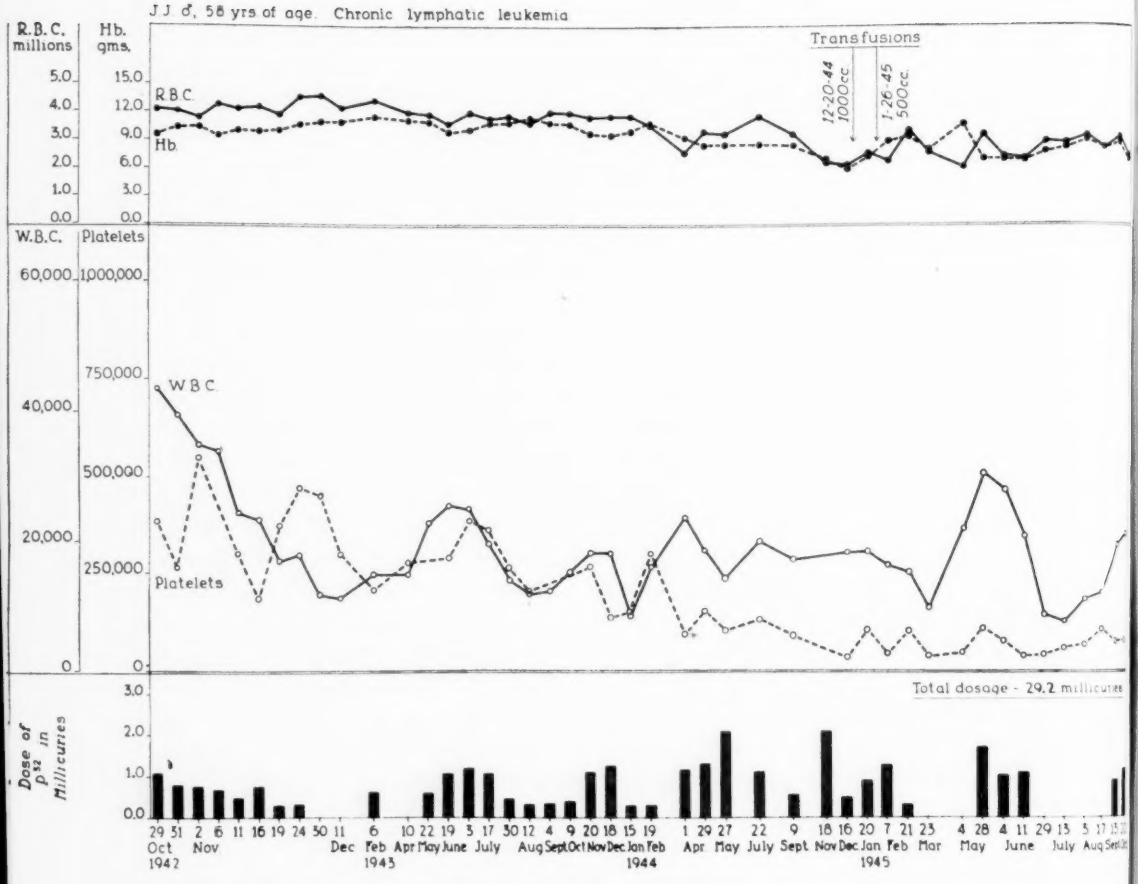

Fig. 11.—Chronic lymphatic leukemia. Response to P^{32} therapy.

Case Summary.—J. J., a man 58 years of age, developed weakness and fatigability in August, 1942, and these symptoms became progressively more severe. In October, 1942, he began to have aching pain in the left upper quadrant of the abdomen. Between June and November, 1942, he lost twelve pounds in weight. There were no other symptoms. He had been a coal miner for almost forty years.

Physical Examination: Patient's hair was white and he looked older than his stated age. There was moderate enlargement of the cervical, axillary, and inguinal lymph nodes (largest nodes, about 1.5 cm. in diameter). There were minimal changes of pulmonary emphysema. The spleen extended 8 cm. below the costal margin; the liver was not palpable.

Laboratory Data: Blood counts (Oct. 29, 1942): leucocytes, 40,300; erythrocytes, 4,060,000; hemoglobin, 9.4 Gm.; reticulocytes, 3.2 per cent; platelets, 382,000; differential: mature lymphocytes, 79 per cent; immature lymphocytes, 2 per cent; segmented polymorphonuclear neutrophiles, 11 per cent; stabs, 4 per cent; eosinophiles, 2 per cent; juveniles, 1 per cent; monocytes, 1 per cent. The sternal bone marrow (Nov. 2, 1942) was hyperplastic; 74 per cent of the white cells were lymphocytes, and there were only 10 nucleated red cells per 100 white cells. Urinalysis was negative. The Kahn reaction was negative.

Course.—About one week after initial injection of P^{32} the patient began to feel stronger, and three weeks later he was almost entirely symptom-free. Two months after treatment was

(Continued on opposite page.)

were thought to have been caused by leukemia infiltration. He also had a severe infection of the gums with Vincent's organisms. The gingivitis healed, and the plaques entirely disappeared following P^{32} therapy. However, it is likely that extraction of all his teeth and local treatment with hydrogen peroxide mouthwashes and gentian violet applications were more important than the radioactive phosphorus in bringing about this improvement.

Two patients (Patients 12 and 13) had extensive skin eruptions diagnosed clinically and pathologically (skin biopsies) as leukemia cutis. In both instances the skin lesions improved markedly but did not disappear entirely following administration of P^{32}. Patient 12 has had several subsequent exacerbations followed by subsidence of the itching eruption.

Mention should be made of Patient 22, who was the outstanding example in this group of the complete failure of radiophosphorus therapy. This patient was a housewife, 53 years of age, who had had weakness and fatigability for seven years. For six months she had noted progressive weakness, progressive swelling of the abdomen with bloating, and marked anorexia. There were several bruises and petechiae scattered over the trunk; there was slight enlargement of the axillary and inguinal lymph nodes; the spleen measured 17.5 cm. below the costal margin and the liver 9 cm. (mid-clavicular line). The total leucocyte count was 310,000 cells per cubic millimeter; 99 per cent of these were lymphocytes. The erythrocyte level was 3,300,000; hemoglobin, 10.9 grams; reticulocytes, 8.0 per cent; and platelets, 152,000. The sternal bone marrow consisted of dense clumps of cells, 92 per cent of which were lymphocytes; practically no nucleated red blood cells were seen. The basal metabolic rate was +35 per cent.

She was given 6.7 millicuries of radiophosphorus over a period of thirty-one days, together with five blood transfusions. There was no improvement in any of her symptoms, and she soon began to bleed from the gums and vagina and into the retinae. The spleen increased slowly in size. The leucocyte level slowly increased, and at the time of death from a cerebral hemorrhage forty-nine days after treatment was started, the leucocyte count was 390,000 cells per cubic

started he was able to return to work in the coal mines (shoveling coal eight hours a day). At this time the lymph nodes were approximately half their former size, and the spleen now measured only 4 cm. below the costal margin. The patient had gained twelve pounds in weight.

He continued to be asymptomatic until July, 1943, when he developed pain in the right hip; at times this was accompanied by severe cramping pains in both thighs and calves. These pains cleared up spontaneously about two months later. Throughout the winter of 1943-1944 he felt quite well and continued to work regularly. In February, 1944, however, the lymph nodes and spleen began to enlarge; by April, 1944, the spleen measured 11 cm. below the costal margin. Toward the end of April he began to tire more easily, and numerous petechiae appeared. In July he became dizzy and began to have nose bleeds. These symptoms persisted, and in January, 1945, he had to quit his job. About this time he began to have severe pains in both shoulders and arms; an x-ray of the cervical spine revealed advanced osteoarthritis. From January to November, 1945, he continued to have intermittent fatigability, petechiae, nosebleeds, oozing from the gums, and occasional pains in the shoulders and hips. On November 17, 1945, the spleen measured 16 cm. below the costal margin.

TABLE 13. HEMATOLOGIC DATA ON FIFTEEN PATIENTS WITH

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	BLOOD COUNTS AT TIME P32 THERAPY WAS STARTED							TOTAL P32 THERAPY ADMINISTERED (MC.)	DURATION OF P32 THERAPY FROM FIRST INJECTION TO LAST INJECTION (DAYS)	BLOOD COUNTS AFTER CYTE COUNT		
			W.B.C. (PER C.MM.)	R.B.C. (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% LEUCOSARCOMA CELLS	% NORMAL LYMPHOCYTES	% POLYMORPHONUCLEAR NEUTROPHILES (INC. STABS)			W.B.C. (PER C.MM.)	R.B.C.[c] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)
1	16, F	X-R	74,000	2.40	7.7	14,000	90	4	0	5.258	24	15,900	3.00 T-3	8.9
2	39, F	S	466,000	2.23	8.8	102,000	97	0	1	25.800	184	16,350	4.16 T-1	13.3
3	20, M	X-R, T	12,750	1.31	4.1	10,000	94	1	5	2.227	13	12.800	2.52 T-5	6.3
4	37, F	T	1,650	3.35	12.0	117,000	62	18	18	23.739	720	6,400	3.65	13.7
5	28, F	X-R, Fo	40,400	1.26	4.5	38,000	99	0	1	4.530	22	8,250	1.89 T-3	5.8
6	74, F	None	1,100	3.59	10.4	431,000	14	25	55	6.808	44	1,350	3.33	9.5
7	17, M	None	8,450	3.36	9.0	302,000	75	6	17	13.205	71	2,550	2.90	8.2
8	25, F	T	500	3.45	10.7	566,000	99	0	0	6.883	33	3,950	4.84 T-1	13.8
9	55, F	None	258,000	3.03	8.6	24,000	98	1	1	17.256	102	6,000	3.04 T-1	7.7
10	35, M	None	20,050	3.12	7.0	62,000	75	6	10	12.157	91	4,100	3.73	12.3
11	9, M	X-R	97,000	4.01	12.8	377,000	85	0	7	10.088	28	10,600	2.75 T-1	8.1
12	13, M	T	15,600	4.40	13.7	16,000	90	4	5	6.858	28	5,500	3.49	10.5
13	49, M	X-R	4,500	4.37	14.2	490,000	0[d]	26	59	11.035	156	5,500	4.09	12.5
14	55, F	None	66,000	2.77	7.4	380,000	96	0	2	2.755	3	5,400	1.60 T-1	5.6
15	44, F	None	3,450	1.43	4.4	108,000	18	44	30	9.560	45	3,200	2.55 T-3	7.3

[a]X-R = X-ray therapy; S = sulfathiazole; T = transfusion; Fo = Fowler's solution.

[b]If the total leucocyte count became progressively more abnormal during the period of treatment the counts given in these columns represent the last counts prior to the terminal rapid rise (if leucocytosis) or fall (if leucopenia) of leucocyte count.

millimeter, of which 99 per cent were lymphocytes. Post-mortem examination revealed the usual lesions of lymphatic leukemia in the liver, in the spleen, and in all lymph nodes studied and extensive replacement of the bone marrow by lymphocytes. Only a few scattered foci of myelopoiesis and erythropoiesis were found in the marrow. The viscera were studded with petechiae, and there was extensive hemorrhage both into the ventricles of the brain and into the subarachnoid space. This case illustrates the fact that no benefit can be anticipated from P^{32} therapy in patients when the normal marrow tissues have been extensively and irreparably destroyed.

Two patients with chronic lymphatic leukemia who responded in a characteristic manner to therapy with P^{32} are illustrated in Figs. 10 and 11. In both of these patients a hematologic remission was obtained, and symptoms were ameliorated following treatment.

2. *Leucosarcoma or Acute Lymphatic Leukemia:* Of the fifteen patients with leucosarcoma included in this study, six were men and nine were women; all of them have died. The duration of symptoms prior to therapy with P^{32} varied from one to sixteen months (excluding Patient 13, in whom the duration was uncertain); the average duration was five months.

Five of the patients had had previous x-ray therapy. In Patient 3 (Table 13) this had produced no symptomatic improvement of any sort. In two other

LEUCOSARCOMA TREATED WITH RADIOACTIVE PHOSPHORUS

P32 THERAPY (ON DAY TOTAL LEUCO- WAS MOST NEARLY NORMAL[b]				BLOOD COUNTS IMMEDIATELY PRIOR TO DEATH						DURATION OF DISEASE FROM FIRST SYMPTOM		
PLATELETS (PER C.MM.)	% LEUCOSARCOMA CELLS	% NORMAL LYMPHOCYTES	% POLYMORPHONUCLEAR NEUTROPHILES (INC. STABS)	W.B.C. (PER C.MM.)	R.B.C.[c] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% LEUCOSARCOMA CELLS	% NORMAL LYMPHOCYTES	% POLYMORPHONUCLEAR NEUTROPHILES (INC. STABS)	TO START OF P32 (WK.)	TO DEATH (WK.)
21,000	71	23	4	15,900	2.21 T-5	6.5	2,000	98	1	0	16	20
667,000	90	0	7	23,100	1.23 T-1	4.7	1,000	100	0	0	30	56
32,000	96	1	1	550	1.10 T-5	3.6	4,000	50	30	10	14	16½
818,000	0	25	54	43,500	2.51 T-32	6.3	7,000	85	1	4	36	140
19,000	81	1	10	350	1.44 T-6	3.9	6,000	90	0	10	64	68
694,000	8	40	44	300	2.46 T-1	7.6	52,000	0	10	20	26	33
162,000	0	58	42	750	2.82 T-5	10.2	25,000	76	0	16	24	34
778,000	14	66	14	35,850	3.58 T-1	10.3	18,000	95	1	4	6	12
24,000	43	18	5	3,650	.63 T-10	2.3	6,000	98	2	0	16	30
498,000	6	16	68	80,000	2.36	7.5	38,000	95	0	4	12	24
27,000	77	5	16	5,550	2.31 T-3	6.5	46,000	93	5	0	5	9
210,000	86	3	7	350	1.67 T-4	5.0	21,000	44	44	0	29	**33**
---	0	25	71	61,400	3.76 T-10	10.1	98,000	99	0	1	416[e]	**421**
4,000	76	12	10	5,400	1.60 T-1	5.6	4,000	76	12	10	4	5½
127,000	0	82	10	850	2.08 T-6	5.1	1,000	68	32	0	16	23½

[c]T = Transfusions. The figure indicates the number of transfusions given.

[d]Diagnosis of leucosarcoma was made on the basis of bone marrow examination (50% leucosarcoma cells).

[e]Duration of lymphosarcoma.

instances, x-radiation had been followed by marked symptomatic improvement lasting one month (Patient 1) and five months (Patient 5). The fourth patient (Patient 11) was asymptomatic and was treated with x-radiation solely because he had developed enlarged nodes in the neck; following treatment these nodes decreased somewhat in size. The last of these patients (Patient 13) was a physician who had a remarkable history. Eight years prior to the time we first saw him he noted enlarged lymph nodes in the axillae and inguinal regions. A biopsy was performed in another city, and the diagnosis of lymphosarcoma was made.* X-ray therapy was given, and the lymph node enlargement completely disappeared. The patient remained subjectively well until four and one-half years later when he developed a lump in the left upper quadrant which was thought to be spleen. X-radiation was followed by complete disappearance of the mass. On both of these occasions repeated blood studies revealed no cytologic abnormalities. When we first saw the patient he still had perfectly normal leucocyte, erythrocyte, and differential counts; the diagnosis of leucosarcoma was established when 50 per cent of the cells in the sternal marrow were found to be leucosarcoma cells. Terminally, marked anemia, thrombocytopenia, and a moderate leucocytosis occurred; 99 per cent of the cells in the peripheral blood were leucosarcoma lymphocytes.

*The lymph node sections were studied by Dr. Robert Moore, Professor of Pathology, Washington University School of Medicine, who confirmed the diagnosis.

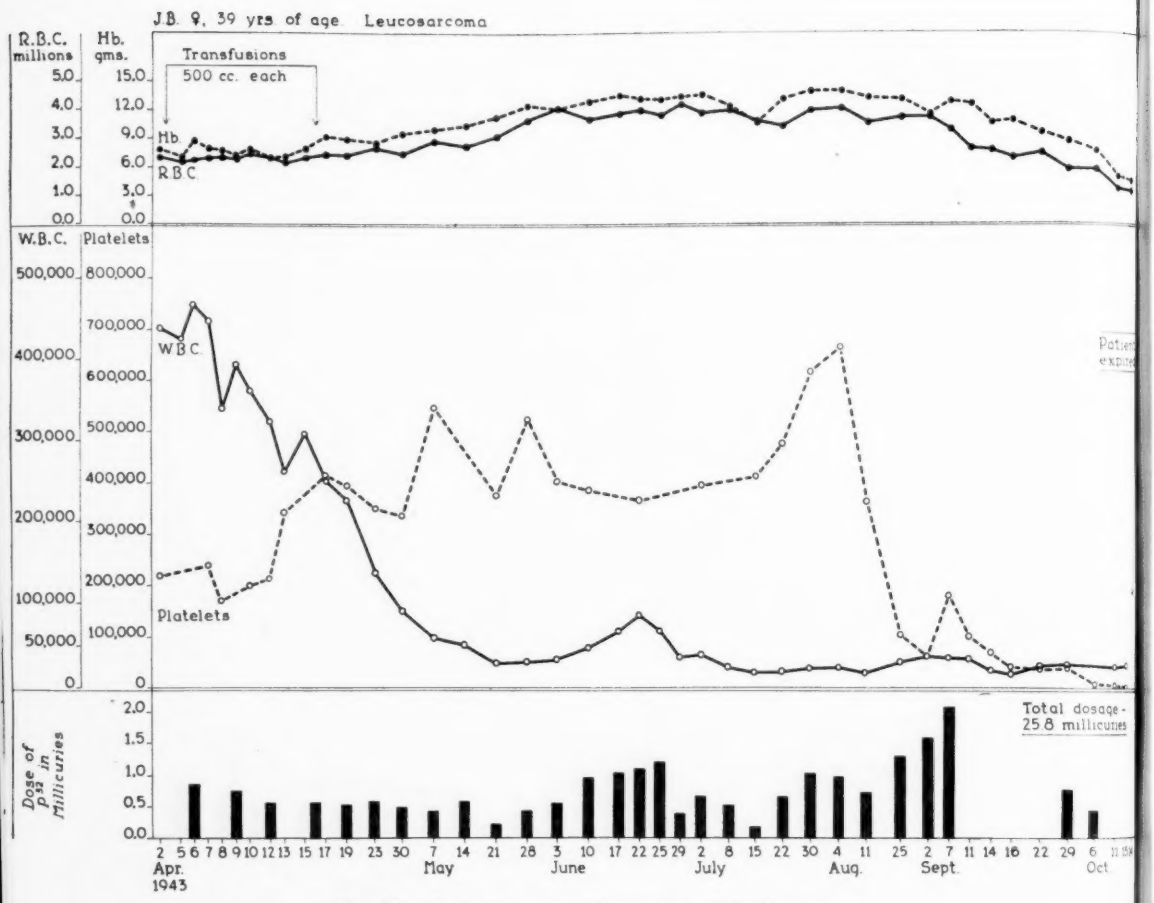

Fig. 12.—Leucosarcoma. Response to P^{32} therapy.

Case Summary.—J. B., a housewife, 39 years of age, first noted moderate fatigability in July, 1942; this progressed very slowly. In October, 1942, the ankles began to swell and she noted that she was becoming pale. In January she had bleeding of the gums, but this lasted only a few days. On March 9, 1943, she developed a severe sore throat with chills and fever. She was given sulfathiazole. Severe sore throat and high fever persisted. She became progressively weaker, and during the month of March she lost ten pounds. We first saw her on April 2, 1943.

Physical Examination: The patient was pale and listless. There were several large purpuric spots on the right thigh. The tonsils were large and fiery red, and there was a gray ulcerated area on the left tonsil. Small shotty nodes ranging up to 1 cm. in diameter were felt in the neck and both axillae. The spleen extended 18 cm. below the costal margin. A small amount of blood was seen coming from the cervical os.

Laboratory Data: Blood counts (April 2, 1943): leucocytes, 439,000; erythrocytes, 2,350,000; hemoglobin, 7.8 Gm.; reticulocytes, 1.0 per cent; platelets, 216,000; differential: 97 per cent typical leucosarcoma cells. The sternal bone marrow was hyperplastic, and 95 per cent of the white blood cells were leucosarcoma cells; 10 per cent of these were in mitosis. Fifteen normoblasts and 9 erythroblasts were seen per 100 myeloid cells. The urine was

(Continued on opposite page.)

TABLE 14. ANALYSIS OF SYMPTOMATIC RESPONSE TO P^{32} THERAPY OF FIVE PATIENTS WITH LEUCOSARCOMA

PATIENT	SYMPTOM BEFORE P^{32} THERAPY	MAXIMUM IMPROVEMENT IN SYMPTOMS	DURATION OF SYMPTOMATIC REMISSIONS	OTHER THERAPY
5	Profound weakness, severe soreness of gums, chills, anorexia	Anorexia disappeared entirely; all other symptoms improved	2 wk.	Blood transfusions
8	Severe sore throat, marked malaise, chills and feverishness, slight weakness	All symptoms completely relieved	3 wk.	One transfusion; severe pharyngitis and tonsillitis treated with penicillin
9	Marked weakness, profuse sweating, chills, moderate dyspnea and anorexia	All symptoms completely relieved except weakness	2 mo.	Transfusions; left lower lobe pneumonia was treated with sulfadiazine
12	Marked weakness and anorexia, severe pain in extremities, chills and feverishness	Pains in extremities completely cleared up and all other symptoms lessened in severity	3 wk.	None
15	Marked weakness, dyspnea, anorexia, severe pain in extremities, moderate cough and pain in chest	Moderate lessening in severity of all symptoms	1 wk.	Sulfadiazine, codeine, six transfusions

Various dosages of radioactive phosphorus were tried in the treatment of these patients. As noted in Table 13, the total dosage administered ranged from 2.7 to 25.8 millicuries; the P^{32} was usually given in relatively small amounts (0.5 to 1.0 millicuries) at fairly frequent intervals (once every two to five days). Since many of the patients had either normal leucocyte levels or a leucopenia when therapy was started, the white blood cell count was of little value as a guide to therapy. An attempt was made to regulate the dosage by serial bone marrow studies. Treatment was temporarily discontinued whenever a precipitous fall in the erythrocyte or thrombocyte levels occurred.

negative. Kahn reaction, negative. A swab from the throat revealed tremendous numbers of Vincent's organisms. On throat culture, the predominating organism was a pneumococcus.

Course: On April 4, 1943, sulfadiazine therapy was resumed (patient had been off the drug for three days); the sulfadiazine blood level was maintained between 5 and 10 mg. per cent for the next week, after which the dosage was decreased to 0.5 Gm. four times a day for another two weeks. P^{32} therapy was also started on April 4, 1943. The patient began to feel stronger about April 9, and the temperature gradually dropped until April 14, when it became normal. The spleen decreased in size, and on April 16 it measured 12 cm. below the costal margin. Her throat continued to be sore until April 25. Her strength gradually improved until about May 15, 1943, at which time she stated she felt perfectly well. She continued to be entirely asymptomatic until September 1. On June 10, 1943, the spleen extended 4.5 cm. below the costal margin. Early in September she again began to feel tired. About Sept. 9, 1943, she developed coryza, a cough, and pain under the sternum; she was again given sulfadiazine. These symptoms subsided, but early in October she developed increasing weakness, pain in the back, diarrhea, swollen tender gums, and fever. On Oct. 12, 1943, the diagnosis of right lower lobe pneumonia was made, and patient expired on Oct. 14, 1945. At post-mortem examination there was marked infiltration of all organs examined (except the pancreas) with leucosarcoma cells.

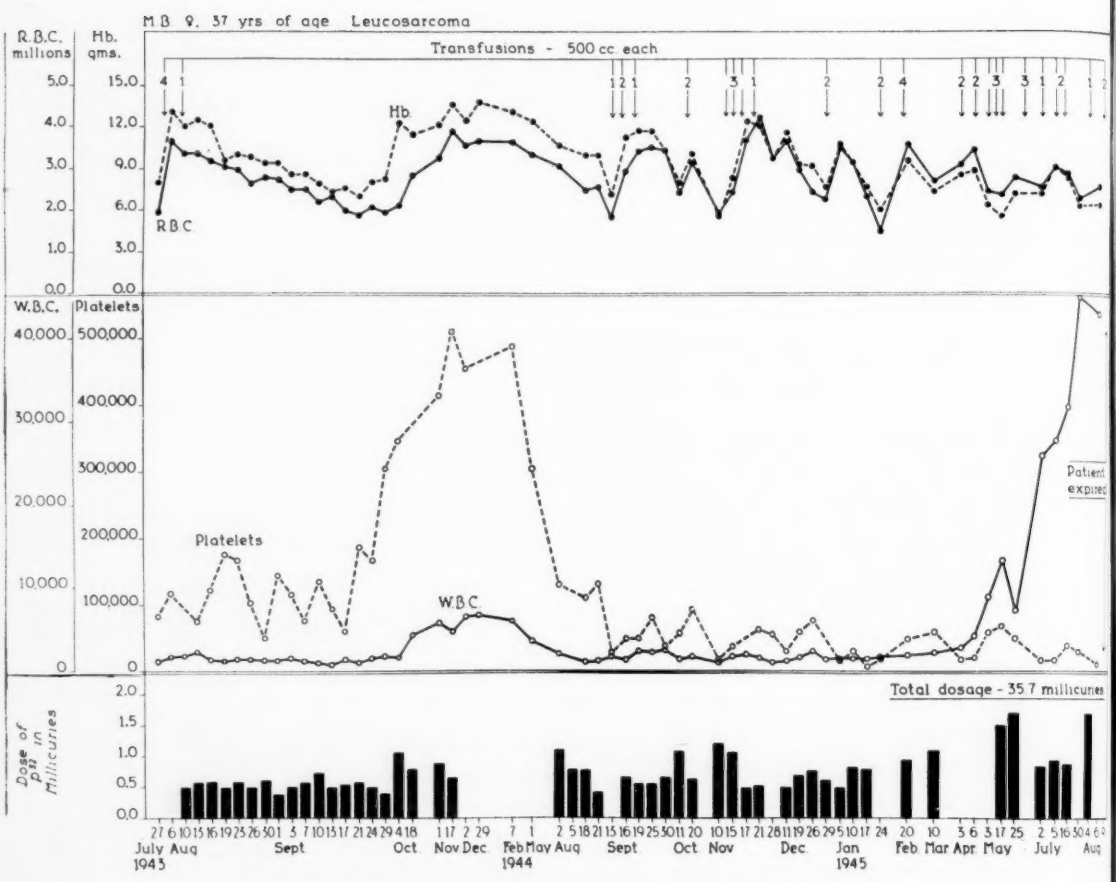

Fig. 13.—Leucosarcoma. Hematologic response to P^{32} therapy.

Case Summary.—M. B., a housewife, 37 years of age, first noted moderate fatigability in August, 1942. This did not progress until February, 1943, when she became rapidly weaker and the ankles began to swell. She was hospitalized on March 7, 1943. Physical examination was negative except for pallor and a low-grade fever. The total leucocyte count was 1,000 cells per cubic millimeter; erythrocytes, 1,600,000; and platelets, 100,000. The bone marrow was found to be hyperplastic, and 94 per cent of the cells in the marrow were leucosarcoma cells. She was given eleven blood transfusions and was discharged May 23 without any other therapy. She was readmitted July 26, 1943, because she had developed a large perirectal abscess. She was too weak even to sit up in bed.

Physical Examination: The skin was strikingly pale. There was a large, red, hot, exquisitely tender area just to the left of the anus. There were no petechiae or ecchymoses, and the lymph nodes, liver, and spleen were not palpable.

Laboratory Data: Blood (August 10, 1943): leucocytes, 1,600; erythrocytes, 3,350,000; hemoglobin, 12.0 Gm.; reticulocytes, 1.8 per cent; platelets, 116,000; differential: polymorphonuclear neutrophiles, 10 per cent; stabs, 8 per cent; monocytes, 2 per cent; adult, small lymphocytes, 18 per cent; leucosarcoma cells, 62 per cent. A second sternal bone marrow examination revealed 87 per cent leucosarcoma cells.

Course: The rectal abscess was incised and sulfamerazine therapy was started on Aug. 6, 1943. She received six blood transfusions. On August 10, therapy with radioactive

(Continued on opposite page.)

The cytologic changes in the blood following radiophosphorus therapy are summarized in Table 13. The leucocyte level dropped in all patients except two who initially had marked leucopenia. In the latter two instances, the leucocyte level increased progressively (to normal in Patient 4 and to 35,000 cells per cubic millimeters in Patient 8). All of these patients except one (Patient 10) received blood transfusions, in spite of which the erythrocyte level slowly fell in all but two. Nine of the subjects had an initial thrombocyte level of less than 300,000 per cubic millimeter; in all fifteen patients the platelet count was below 100,000 per cubic millimeter just prior to death, and in every instance but one the level was considerably lower than the pretherapy figure.

Eight patients in this group showed no significant improvement in any of their symptoms following therapy with P^{32}, and in most of them there was a rapid progression of all symptoms. In seven patients there was definite symptomatic improvement; four of these individuals became almost entirely symptom-free. However, in all but two the remission was of brief duration and could well have been due to transfusions, chemotherapy, bed rest in the hospital, or psychologic factors. The symptomatic responses of the five patients who had very brief improvement are analyzed in Table 14; the two patients who had more prolonged symptomatic remissions are not included in this table, as data on these individuals are given in detail in Figs. 12 and 13. Even in these

phophorus was instituted. One month later, at a time when the patient had received 5.78 millicuries of radioactive phosphorus, the total white count was 659; only 20 per cent of these were leucosarcoma cells, whereas prior to treatment 62 per cent of the cells had been leucosarcoma cells. In spite of the profound leucopenia, the patient felt considerably stronger and the fever began to subside. During the next two weeks clinical improvement was progressive, weakness disappeared, and appetite became tremendous. She was discharged from the hospital seven weeks after treatment was started, at which time the total leucocyte count was just beginning to increase. Twelve weeks after treatment was started the total leucocyte count was 5,500 and less than 1 per cent of the cells in the peripheral blood were abnormal lymphocytes which we classified as leucosarcoma cells. A third bone marrow examination was done on the forty-sixth day of treatment, at a time when the total leucocyte count was only 1,200. The marrow showed a striking increase in myeloid and erythroid cells, and only 20 per cent of the cells were of the lymphoid series. Treatment was continued for three and one-half months; 11.9 millicuries of radioactive phosphorus were given. By this time the total leucocyte count, the differential, and the platelet count were all perfectly normal, and the red blood cell count had come up to 3,900,000 per cubic millimeter, with 13.6 Gm. of hemoglobin. The patient received no further therapy and the blood counts remained within normal limits for a period of five and one-half months. The white count then began to fall progressively and her symptoms returned; a second course of treatment was begun in August, 1944. She again improved symptomatically but the leucocyte count did not rise. She continued to have periods of relative freedom from symptoms alternating with periods of weakness, anorexia, and bleeding from the gums. She received occasional blood transfusions as indicated in the chart. In April, 1945, concomitant with a rise in the leucocyte count, she became weaker, began to bleed from the gums, and developed diarrhea, cramping abdominal pain, fever, and an abscess on her buttock. On May 17, 1945, sulfadiazine therapy was started. In June, 1945, extensive ecchymoses appeared over her body. The symptoms fluctuated in severity until July, 1945, when she became rapidly worse with increasing fever, anorexia, nausea and vomiting, and pain in the chest. She was found to have bilateral bronchopneumonia. On July 25, 1945, the spleen was palpable for the first time. She expired Aug. 9, 1945, exactly two years after treatment with P^{32} was started.

latter two instances where prolonged symptomatic and hematologic improvement occurred, other therapeutic measures were employed which certainly accounted for some of the improvement noted.

The changes which occurred in the physical signs following administration of P^{32} can be summarized very briefly. Of the fifteen patients, seven had ecchymoses or petechiae in the skin when first seen; in two of these individuals, this manifestation became much less marked and in the other five there was no improvement. Two individuals showed hemorrhages in the mucous membranes and retinae; these disappeared in one and became steadily worse in the other. Eleven patients had enlarged lymph nodes; these became no longer palpable in one patient and decreased in size in the other ten. Eleven subjects had an initial splenomegaly; the spleen became no longer palpable in one, decreased in four, and increased progressively in the other six. Hepatomegaly, originally present in six patients, showed slight improvement in three and no diminution in the other three.

C. *Comparison of Radioactive Phosphorus With Other Forms of Therapy Used in the Treatment of Lymphatic Leukemia.*—The therapeutic effectiveness of P^{32} should be compared principally with x-radiation because Fowler's solution is a relatively inferior therapeutic agent for the treatment of chronic lymphatic leukemia. The hematologic remissions produced by administration of P^{32} are incomplete in that the percentage of lymphocytes does not regularly return to normal even though the total white blood cell count frequently does. A similar statement may be made for x-ray therapy. Radioactive phosphorus is no more effective than x-radiation in relieving symptoms of patients with chronic lymphatic leukemia and often does not produce as much decrease in the size of lymph nodes and spleen as does x-ray. In none of our patients did P^{32} induce so complete a remission that further injections could be withheld for as long as a year. The remissions, therefore, were no better and may not have been as prolonged as is usual with x-radiation. Administration of radioactive phosphorus has one advantage in that it does not cause radiation sickness.

There was no indication that P^{32} is likely to prolong life more than does x-ray. The duration of symptoms to death or to Sept. 1, 1945, for the twenty-three patients who have been followed for a year or more (Patients 1 to 21, 23, and 24) was 31.8 months; nine of these persons are still living. They have been treated with P^{32} for an average of 14.4 months. The nine living patients in this group had had symptoms for an average of 12.4 months at the time P^{32} was first given and had an average duration of their disease on Sept. 1, 1945, of 36.3 months. When these figures are compared with the average duration of 3.29 years found for 152 cases of chronic lymphatic leukemia,[48] it is clear that the final figures for the duration of the disease in patients treated with P^{32} are not likely to exceed them by a significant margin. Minot and his associates[50] reported that the course of the disease was not appreciably lengthened by x-ray therapy.

Results in the treatment of acute lymphatic leukemia (including leucosarcoma) have been as disappointing as with all forms of therapy. An occa-

sional patient does have a relatively long, incomplete remission, but these may occur spontaneously and cannot yet be attributed to the therapeutic effectiveness of P^{32}.

D. *Summary and Recommended Method for Administering P^{32} to Patients With Lymphatic Leukemia.*—In our experience, somewhat smaller doses of radioactive phosphorus suffice to restore the total leucocyte level to normal in chronic lymphatic leukemia than in chronic myelogenous leukemia. However, the percentage of lymphocytes dropped below 50 per cent, and the percentage of granulocytes rose above 50 per cent in only sixteen of the thirty patients. Furthermore, a rise in the erythrocyte and platelet levels following P^{32} therapy was less frequent and much less marked among the patients with chronic lymphatic leukemia than among those treated for myelogenous leukemia. In addition to the less favorable hematologic response, symptoms and physical signs also responded less favorably in the individuals with lymphatic leukemia.

The chief advantage of radioactive phosphorus therapy over x-radiation in the treatment of chronic lymphatic leukemia is freedom from radiation sickness. It is our impression that therapy with P^{32} is probably as satisfactory as, but no better than, x-radiation in relieving symptoms and in prolonging life.

In general, the acute varieties of lymphatic leukemia are not significantly benefited by radioactive phosphorus. The few remissions observed could well have been due to blood transfusions, chemotherapy of infections, or could even have been spontaneous remissions.

The same schedule of therapy recommended for myelogenous leukemia can be employed for lymphatic leukemia except that, as a rule, administration should be discontinued earlier because of the more rapid fall in the leucocyte level in patients with lymphatic leukemia.

Seven of our patients with chronic lymphatic leukemia had an initial white blood cell level below 18,000 cells per cubic millimeter. Regulation of therapy for these individuals was more difficult. The severity of the disease in such individuals must be determined by the bone marrow cytology and the degree of associated anemia and thrombocytopenia. It is our feeling that roughly comparable dosages of P^{32} should be employed in the management of leucopenic leukemia as would be administered to patients with elevated leucocyte levels. An intelligent decision as to when therapy should be discontinued can be reached only by frequent determinations of the blood erythrocyte, platelet, and segmented polymorphonuclear levels and by serial bone marrow studies.

VI. MONOCYTIC LEUKEMIA

A. *Review of the Literature.*—The response of six patients with monocytic leukemia to therapy with P^{32} has been reported.[1, 5, 15] Specific data are given for only three of these patients.

Lawrence and his associates[1] treated a 24-year-old woman who complained of progressive weakness of four years' duration, swelling of the lymph nodes of the neck, and furuncles on the forehead of three months' duration. Significant physical findings included pallor, generalized lymph node enlargement, herpetic lesions on the lips, bleeding of the gums, and a smooth liver edge palpable at the costal margin. When she was admitted to the hospital (Dec. 24, 1937), the total erythrocyte count was 2,400,000; hemoglobin, 60 per cent; leucocytes, 11,950, with 21 per cent polymorphonuclear cells, 22 per cent lymphocytes, and 50 per cent monocytes.

Treatment with radioactive phosphorus was started Jan. 13, 1938. By that time the white blood cell level had increased to 58,000. Between January 13 and February 18, the patient was given orally nine doses of radiophosphorus totaling 23.2 millicuries. The furuncles were incised and drained. She was also given iron by mouth and four blood transfusions. The leucocyte level rose during the first week of therapy to about 178,000 cells per cubic millimeter and then fell rapidly to approximately 15,000 cells, most of which were monocytes. New furuncles appeared, and stomatitis with ulceration and hypertrophy of the gums became progressively more severe. The spleen became easily palpable. Terminally there was a rise in temperature, and the white blood cell level rose to 71,000; 91 per cent of the white cells were monocytes. Death occurred forty-two days after therapy had been started.

Post-mortem examination revealed prominent infiltration of the liver, heart, colon, gall bladder, kidneys, uterus, and ovaries with monocytic cells. The lymph nodes, spleen, and bone marrow were composed largely of monocytes.

Erf and his associates[5] tabulated data on a man, 40 years of age, who had had fatigue, anorexia, and cough for five months and abdominal pain for three weeks. The spleen was felt 1 centimeter below the costal margin and there were hemorrhages in the fundi. The total leucocyte count was 41,000 cells per cubic millimeter. Sternal marrow was hyperplastic and contained "many monoblasts." The patient was given 6.9 millicuries of radiophosphorus by mouth within a period of three days. Death occurred six days after the first dose. Post-mortem examination revealed reticulo-endothelial hyperplasia of most organs, including the kidneys and the submucosa of the gastrointestinal tract.

Warren[15] treated four patients with monocytic leukemia, one of whom was "helped," but the other three showed no response. A case report was given for only one patient, a man, 31 years of age, who had had weakness, anorexia, and dyspnea for two months. The total leucocyte count was 6,500 per cubic millimeter; 50 per cent of the white cells were monoblasts. The patient was given 6.27 millicuries of P^{32} intravenously during a period of three weeks. Death occurred four weeks after the initial injection. Post-mortem examination was interesting in that the bone marrow was hypoplastic; most of the other organs were infiltrated with monocytes.

B. *Analysis of the Results Obtained in Treating Ten Patients With Monocytic Leukemia.*—Of the ten patients with monocytic leukemia whom we have treated with radioactive phosphorus, only one is still living. All of the patients were acutely ill when treatment was started, and eight of them remained in the hospital until they died. Data on these patients are summarized in Table 15. All had had rapidly progressive symptoms including painful bleeding gums, sore throat, fever, anorexia, sore, swollen cervical lymph nodes, and progressive prostration for from ten days to four weeks before admission to the hospital. Patients 3, 5, 7, and 8 felt well until the onset of this period of rapidly progressive symptoms; all of the other patients had noted weakness and fatigability without other manifestations for variable periods ranging up to six months prior to the onset of the acute phase. The average total duration of symptoms was eleven weeks. None of these individuals had been given any previous therapy except for four who had received transfusions. In seven instances transfusions were given during the period of radioactive phosphorus therapy.

The total dosage of P^{32} administered varied from 0.14 millicuries to 11.06 millicuries, with an average of 5.3 millicuries. In every instance except one (Patient 10), treatment was continued until the time of death.

The effect of therapy on the total leucocyte count was variable. The total white blood cell level increased in one-half the cases and decreased in the other

TABLE 15. HEMATOLOGIC DATA ON TEN PATIENTS WITH MONOCYTIC LEUKEMIA TREATED WITH RADIOACTIVE PHOSPHORUS

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	TOTAL P^{32} THERAPY ADMINISTERED (MC.)	DURATION OF P^{32} THERAPY FROM FIRST INJECTION TO LAST INJECTION (DAYS)	BLOOD COUNTS AT TIME P^{32} THERAPY WAS STARTED (ABOVE) BLOOD COUNTS IMMEDIATELY PRIOR TO DEATH (BELOW)[b]							DURATION OF DISEASE FROM FIRST SYMPTOM	
					W.B.C. (PER C.MM.)	R.B.C.[c] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% MONOBLASTS AND YOUNG MONOCYTES	% MONOCYTES (MATURE)	% GRANULOCYTES (POLYS AND STABS)	TO START OF P^{32} (WK.)	TO DEATH (WK.)
1	40, F	None	0.142	2	49,000	2.11	5.9	8,000	84	2	0	14	14⅓
					90,000	1.82	5.6	40,000	86	6	0		
2	65, F	None	2.793	71	8,500	1.25	4.7	73,000	25	50	12	12	23
					86,000	2.10 T-3	6.9	118,000	90	0	2		
3	56, M	T	1.780	3	82,000	1.92	6.9	12,000	70	7	6	1½	2
					73,500	2.17	6.2	15,000	83	0	9		
4	25, M	T	5.210	16	143,000	2.03	6.3	24,000	70	0	14	28	30½
					84,000	2.39 T-3	8.0	62,000	32	53	3		
5	53, F	None	11.056	62	18,200	1.56	5.5	58,000	31	49	1	4	14
					11,300	1.59 T-16	4.4	10,000	97	1	0		
6	69, F	T	4.964	8	87,500	2.30	7.8	69,000	94	3	1	12	14
					169,000	2.86 T-1	7.9	320,000	93	4	0		
7	62, M	None	4.504	4	72,500	1.72	6.4	20,000	91	2	0	6†	7
					100,000	1.10 T-1	4.4	11,000	90	0	0		
8	60, F	None	7.658	12	25,400	1.98	8.0	416,000	45	21	13	1½	3½
					161,000	2.26 T-2	8.0	34,000	93	0	3		
9	50, F	None	6.305	8	103,000	3.45	10.4	82,000	47	34	5	8	9½
					95,000	2.42	7.8	19,000	8	80	5		
10	64, M	T	8.720	62	45,500	2.14	8.2	218,000	0	81	2	24	35+
					6,200	3.66 T-13	11.7	223,000	43	6	35		

[a]T = Transfusion of whole blood.

[b]Patient 10 was still living at the time this chart was prepared; the counts in these columns for Patient 10 are the last counts obtained eighty days after treatment was started.

[c]T = Transfusions. The figure indicates the number of transfusions given.

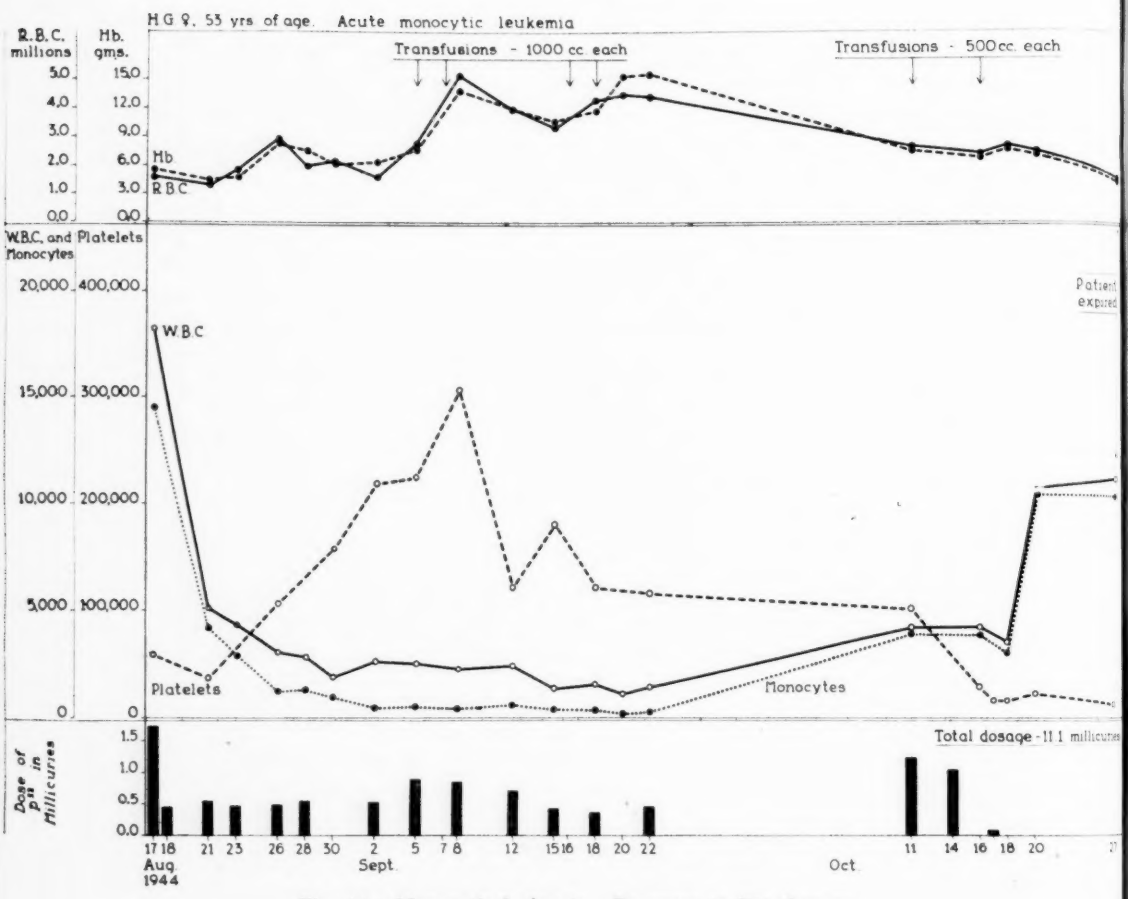

Fig. 14.—Monocytic leukemia. Response to P^{32} therapy.

Case Summary.—H. G., a housewife, 53 years of age, was in excellent health until July, 1944, at which time she developed pain in the lower jaw. The patient had many carious teeth and she attributed the pain to a toothache. However, within a few days the gums of both upper and lower jaws became swollen, red, and tender. Shortly thereafter she developed a sore throat, swelling of the glands in the neck, and fever. She began to have generalized headaches, spells of faintness, and increasing weakness. She was given sulfadiazine therapy (amount unknown) without improvement. In August, 1945, penicillin therapy was started; she was unimproved two days later when we first saw her.

Physical Examination: Blood pressure was 150/80. Patient was an obese, pale, acutely ill, white woman. There was marked hypertrophy of the gums with necrosis and ulceration, especially around the lower anterior teeth which were carious and loose. There was an elevated purplish area in the soft palate. The left anterior tonsillar pillar was ulcerated. The breath was foul. The submaxillary nodes were large, firm, and moderately tender; there was no other lymph node enlargement. There was an irregular area of hemorrhage in the right fundus. The liver and spleen were not palpable.

Laboratory Data: Blood counts (Aug. 17, 1945): leucocytes, 18,150; erythrocytes, 1,560,000; hemoglobin, 5.5 Gm.; reticulocytes, 0.5 per cent; platelets, 58,000; differential: stabs, 1 per cent; eosinophiles, 2 per cent; basophiles, 1 per cent; juveniles, 4 per cent; myelocytes, 30 per cent; lymphocytes, 9 per cent; mature monocytes, 49 per cent; immature

(*Continued on opposite page.*)

half. Furthermore, in all the patients who have died, the percentage of monocytic cells in the blood was greater just prior to death than at the time treatment was started. No conclusions can be drawn regarding the effect of P^{32} on the erythrocyte level since transfusions were given in all but three instances. The thrombocyte level increased in four patients, decreased in three, and showed no significant change in three. More detailed hematologic data on these patients are given in Table 15.

There was no definite evidence that any of these patients were benefited symptomatically by P^{32} therapy. Weakness temporarily became less severe in three instances (Patients 2, 5, and 10), but transfusions had been given to them repeatedly, and it seems likely that this was the factor responsible for the increase in strength. The two other most common symptoms were hemorrhagic manifestations (present in eight instances) and soreness of the gums (four instances). Both of these symptoms decreased in severity in Patient 5, but neither of them was significantly relieved in any of the other patients.

The physical signs likewise were not favorably influenced. At the time therapy with P^{32} was started, the lymph nodes were slightly enlarged in six patients, the spleen was palpable in five, and the gums were hypertrophied in four. In no instances was there any improvement in any of these abnormalities.

Hematologic data and the case report for Patient 5 are shown in Fig. 14. The response of this woman to therapy was not typical in that she lived longer than the average after treatment was started and showed somewhat more symptomatic improvement than did most of the patients in this group.

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monocytes, 25 per cent; monoblasts, 6 per cent. A few normoblasts were seen in the peripheral blood. The sternal bone marrow differential (Aug. 17, 1944) was: stabs, 3 per cent; juveniles, 8 per cent; "C" myelocytes, 9 per cent; "B" myelocytes, 4 per cent; immature monocytes, 52 per cent; monoblasts, 10 per cent; primitives, 13 per cent; plasma cells, 1 per cent. There were only 4 nucleated red blood cells per 100 myeloid cells. The urine was negative. The Kahn reaction was negative.

Course: Sulfadiazine and penicillin were discontinued on Aug. 15, 1944. On P^{32} therapy and transfusions she gradually improved. The temperature spiked daily to 39° C. until August 29, after which it dropped to lower levels. The ulcer of the tonsillar pillar healed and gums became much less sore; the hypertrophy and redness of the gums improved. On September 12 the temperature spiked to 40.4° C., and she developed a headache and marked restlessness. She became nauseated and vomited repeatedly. By September 22 these symptoms had subsided, and she felt so much stronger that she was discharged from the hospital. Two days after returning home she developed profuse vaginal bleeding and bleeding from the gums; this lasted several days. About October 1 she had severe epigastric pain followed by nausea and vomiting. The temperature began to rise. These symptoms persisted and she developed a rapidly spreading furunculosis over her back. She became semistuporous on October 17, and purpura and bleeding from the gums increased. She expired Oct. 27, 1944.

Post-mortem examination revealed extensive leukemic infiltration of the bone marrow, lymph nodes, lungs, gingivae, pancreas, liver, spleen, kidneys, adrenals, and uterus. The bone marrow was hyperplastic, the vast majority of the cells being monocytes; myeloid and erythroid elements were present but greatly decreased in numbers, and no megakaryocytes were seen. There were areas in the bone marrow in which the cells stained very faintly and were apparently undergoing early necrosis.

C. *Summary.*—Analysis of the hematologic and clinical data on our ten patients confirms the impression of previous investigators that radioactive phosphorus is of no value in the treatment of monocytic leukemia.

VII. HODGKIN'S DISEASE

A. *Review of the Literature.*—Four reports[10, 12, 15, 17] have been published describing the results of therapy with radioactive phosphorus in thirty-eight patients with Hodgkin's disease. No case reports or data are given for any of these individuals except for the eleven patients studied by Hoster and Doan.[17]

Fitz-Hugh and Hodes[10] treated five persons, only one of whom was improved following P^{32} therapy. This was a patient who was becoming x-ray-fast after two years of treatment. The other four patients were not benefited; three of them had received roentgen therapy previously, and the fourth patient had a huge mediastinal mass which did not respond to P^{32} but did regress following intensive x-radiation.

Low-Beer and co-workers[12] observed no satisfactory results in the nineteen patients they treated. Temporary improvement occurred only in those individuals who had mild symptoms of short duration, and in no case was the duration of remission more than one year.

Only one of the three patients treated with P^{32} by Warren[15] was helped. All three had received x-ray therapy previously.

Of the eleven patients treated by Hoster and Doan,[17] only five had been treated with x-radiation in the past. Radioactive phosphorus was given for from ten to thirty-seven weeks; the average biweekly dose was 2.3 millicuries. All eleven patients showed a decrease in the white blood cell level during the period of P^{32} therapy; the erythrocyte and platelet levels dropped in seven instances. In most cases these evidences of depression of bone marrow activity did not disappear after administration of P^{32} was stopped. Some of the patients developed additional lymph node enlargement during the period of treatment. Temporary benefit occurred in a few instances. X-radiation was given to nine of these patients after the cessation of P^{32} administration, and in every instance but one a favorable response was observed.

B. *Analysis of the Results Obtained in Treating Six Patients With Hodgkin's Disease.*—The six patients with Hodgkin's disease whom we have treated with radioactive phosphorus had had symptoms presumably referable to this disease for from eight to forty-two months. In each of them x-radiation had previously produced symptomatic improvement. Improvement following x-ray therapy had been least striking in Patient 1 of Table 16 (enlarged nodes in the neck decreased somewhat in size, and strength increased but the benefit was of only two weeks' duration); the greatest improvement following x-radiation was noted in Patient 4, who had experienced marked relief of all symptoms following six separate courses of roentgen therapy. All six of these patients are now dead.

The total dosage of P^{32} administered varied from 1.73 millicuries (given within five days) to 13.26 millicuries (administered during a period of seven months). The average total dosage was 8.62 millicuries.

Cytologic changes which occurred in the blood following therapy with P^{32} are shown in Table 16. In four of the six patients (Patients 1 to 4); P^{32} therapy was followed by depression of bone marrow activity, as manifested by significant decreases in the number of leucocytes, erythrocytes, and platelets in the peripheral blood. In two instances (Patients 2 and 3) the platelet count

TABLE 16. HEMATOLOGIC DATA ON SIX PATIENTS WITH HODGKIN'S DISEASE TREATED WITH RADIOACTIVE PHOSPHORUS

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	TOTAL P^{32} THERAPY ADMINISTERED (MC.)	DURATION OF P^{32} THERAPY FROM FIRST INJECTION TO LAST INJECTION (DAYS)	BLOOD COUNTS AT TIME P^{32} THERAPY WAS STARTED (ABOVE) BLOOD COUNTS IMMEDIATELY PRIOR TO DEATH (BELOW)[b]							DURATION OF DISEASE FROM FIRST SYMPTOM	
					W.B.C. (PER C.MM.)	R.B.C.[c] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% MYELOID CELLS[d]	% LYMPHOCYTES	% MONOCYTES	TO START OF P^{32} (MO.)	TO DEATH (MO.)
1	24, M	X-R	11.99	218	3,100	4.13	11.7	677,000	52	21	27	26	34
					1,850	2.69	7.7	94,000	64	18	18		
2	52, F	X-R	9.23	118	14,700	3.69	10.1	790,000	78	16	6	8	16
					11,050	1.35	4.0	2,000	23	77	0		
3	32, F	X-R	5.10	25	5,500	3.18	6.8	782,000	73	12	15	24	25½
					350	2.48 T-4	6.5	20,000	80	10	10		
4	29, M	X-R	13.26	229	4,050	4.16	12.6	715,000	79	12	9	42	59
					3,450	3.18	11.0	235,000	72	22	6		
5	18, F	X-R	1.73	5	9,600	4.53	10.5	861,000	85	9	6	15	17
					9,300	4.65 T-11	11.2	600,000	92	4	4		
6	27, M	X-R	10.41	301	9,800	3.75	12.4	855,000	92	1	7	36	50
					11,250	3.83	9.5	1,140,000	91	3	6		

[a]X-R = X-ray.

[b]The figures in the bottom row represent the blood counts just prior to death (if P^{32} treatment was continued until shortly before death), or one month after the last injection of P^{32} (if treatment was discontinued more than two months prior to death).

[c]T = Transfusions. The figure indicates the number of transfusions given (500 c.c. each).

[d]Includes neutrophilic, eosinophilic, and basophilic cells of all stages of development.

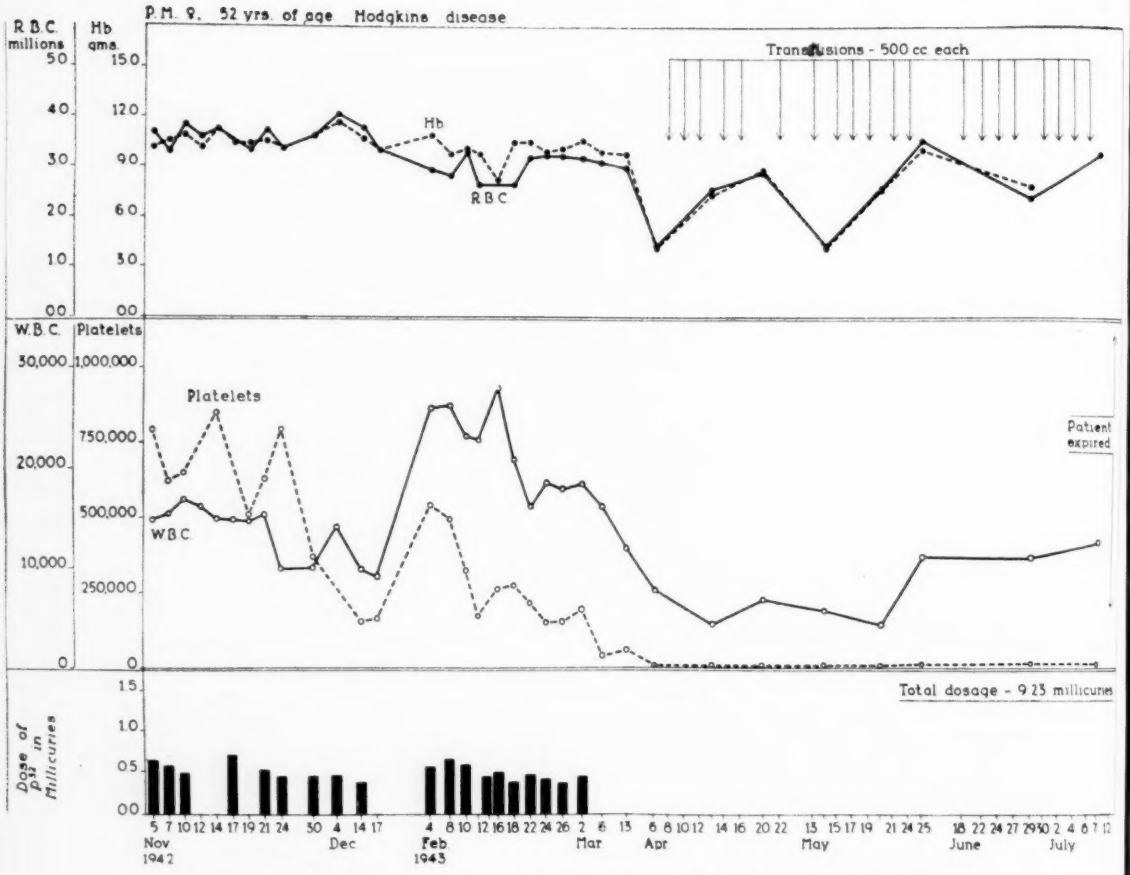

Fig. 15.—Hodgkin's disease. Hematologic changes following P^{32} therapy.

Case Summary.—P. M., a married woman 52 years of age, enjoyed excellent health until August, 1941, when she first noted a dry hacking cough. In May, 1942, the cough became productive of mucoid sputum. About this same time she began to have dull, aching pain in the left lower chest posteriorly. Shortly thereafter she began to have severe night sweats and increasing fatigue. She lost twelve pounds in weight. An x-ray of the chest in June, 1942, showed extensive pulmonary infiltration. Bronchoscopy revealed only chronic inflammation of the bronchi. By August, 1942, enlargement of the axillary and inguinal lymph nodes was apparent; a biopsy (right axillary node) revealed changes typical of Hodgkin's disease. She was given nine x-ray treatments to the right chest and axilla, following which the pain and cough disappeared. She gained nine pounds. During October the nodes in the left groin increased steadily in size and became sore and tender. Radioactive phosphorus therapy was started Nov. 5, 1942.

Physical Examination (Nov. 5, 1942): There was x-ray pigmentation over the right chest and back. A large matted mass of lymph nodes measuring 4 by 3 by 3 cm. was felt in the left inguinal region. Enlarged nodes were also felt in the neck and in the right axilla. The heart and lungs were normal. A firm mass thought to be spleen was felt in the left upper quadrant of the abdomen, but a distinct edge could not be outlined.

Laboratory Data: Urine was negative except for a faint trace of albumin. Kahn test was negative. Blood counts (Nov. 5, 1942): white blood cells, 14,700; red blood cells,

(Continued on opposite page.)

fell to extremely low levels, accompanied in the latter case by widespread subcutaneous hemorrhages. Patient 3 developed a leucopenia of 350 cells per cubic millimeter; a fatal bronchopneumonia then developed. Patients 5 and 6, who did not show these changes in the blood cytology, received such small doses of P^{32} that neither their symptoms nor physical signs showed any improvement following treatment.

Evaluation of the effect of P^{32} on the symptoms of Hodgkin's disease was difficult because of the notorious cyclic fluctuation in the severity of symptoms which occurs even when no treatment is given. Weakness was a prominent symptom in all six of these individuals; following therapy with P^{32} there was some increase in strength in four instances (Patients 1, 2, 4, and 6). The duration of this improvement varied from two weeks (Patient 6) to three months (Patient 2). Chills and fever, initially present in four instances (Patients 1, 3, 4, and 6) were eliminated only in Patient 1. Three patients (Patients 1, 5, and 6) complained of pain in the shoulders, chest, back, or flanks; the pain disappeared entirely in one (Patient 1) and became progressively worse in another (Patient 5). Cough, which was present in two patients (Patients 4 and 5), showed no improvement in either instance.

The physical signs were strikingly unaffected by P^{32}. Four patients (Patients 1, 2, 3, and 5) had enlarged lymph nodes, two (Patients 3 and 4) had splenomegaly, one (Patient 5) had hepatomegaly, and one (Patient 2) had edema of the ankles. None of these physical signs showed any measurable improvement following radiophosphorus therapy, except splenomegaly, which decreased in Patient 4, and lymph node enlargement which decreased in Patient 2.

Hematologic data and the case report for Patient 2 are shown in Fig. 15.

C. *Summary.*—The results obtained when these six patients were treated with P^{32} were similar to those which have been reported by other investigators. From this combined experience, it seems fair to conclude that P^{32} is inferior to x-radiation in the treatment of Hodgkin's disease. Even when the dosage of P^{32} employed is sufficiently large so that serious damage to the bone marrow results, enlarged lymph nodes and splenomegaly are relatively unaffected, and the infrequent symptomatic improvement which has been observed is of such

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3,690,000; hemoglobin, 10.1 Gm.; reticulocytes, 2.8 per cent; platelets, 790,000; differential: eosinophiles 25 per cent; juveniles, 1 per cent; stabs, 4 per cent; segmented polymorphonuclear neutrophiles, 48 per cent; lymphocytes, 16 per cent; and monocytes, 6 per cent. Sternal bone marrow examination showed 43 per cent eosinophiles, 39 per cent lymphocytes, 6 per cent plasma cells, and only 12 per cent cells of the myeloid series. Very few nucleated red blood cells were seen. There were many globules of fat present.

An x-ray of the chest showed considerable increase in the amount of infiltration in each lung.

Course: During the first four months of therapy there was little change in the patient's symptoms. The mass of lymph nodes in the left groin decreased somewhat in size. During March, she became progressively weaker, and by April 7 the extremities were covered with petechiae and ecchymoses. From that time she had constant hemorrhagic manifestations, and the erythrocyte level remained low in spite of twenty blood transfusions. She died eight months after P^{32} therapy was started. A post-mortem examination was not done.

transient duration as to be of no consequence. In only one case in our series was the symptomatic improvement following P^{32} as satisfactory or prolonged as the improvement which the same patient had previously experienced following x-ray therapy.

VIII. LYMPHOSARCOMA, RETICULUM CELL SARCOMA, AND GIANT FOLLICULAR LYMPHOBLASTOMA

A. *Review of the Literature.*—Kenney and his associates[8] in 1942 published data on four patients with lymphosarcoma, two with reticulum cell sarcoma, and two with giant follicular lymphoblastoma treated with radioactive phosphorus. These patients had been given from 70 to 100 microcuries (0.07 to 0.1 millicuries) of P^{32} per kilogram of body weight in divided doses at intervals of from seven to fourteen days. Five patients (two with lymphosarcoma, two with reticulum cell lymphosarcoma, and one with giant follicular lymphoblastoma) had complete remissions for from one to eight months. One patient with lymphosarcoma had complete disappearance of neck nodes and 50 per cent decrease in the size of axillary nodes. Another individual with lymphosarcoma showed initial improvement followed by rapid increase in the size of the nodes. It was felt that this case represented a failure of radioactive phosphorus therapy, as the patient had shown disappearance of lymph node enlargement following small doses of x-ray therapy (300 roentgens) on three previous occasions. The second patient with giant follicular lymphoblastoma apparently had no enlarged lymph nodes, but the spleen was palpable 1 centimeter below the costal margin at the time P^{32} therapy was started; following treatment, the spleen became no longer palpable. These same cases were included in another report by Kenney.[9]

Two of the six patients with lymphosarcoma treated with P^{32} by Fitz-Hugh and Hodes[10] had good remissions. One of these had become x-ray-fast; the clinical improvement which followed radioactive phosphorus therapy was still manifest six months later. Two of the patients were said to have been "benefited" for several months, following which both suffered relapses. One individual with reticulum cell sarcoma was not benefited by moderate doses of P^{32}; he had previously received intensive roentgen radiation and had become "x-ray-fast." No data were recorded.

Low-Beer and co-workers[12] treated thirteen patients with generalized lymphosarcoma, seven of whom were dead at the time of the report. The initial response to therapy was satisfactory. Enlarged nodes usually disappeared, and the general condition of the patients was improved, in some cases returning to normal. The authors made the interesting statement that, "In no patient treated for lymphosarcoma, even though it has been found necessary to build up a high radiation level, has the peripheral blood picture changed appreciably." A case history was given for one of these individuals, a woman, 66 years of age, whose symptoms were of six months' duration. She had received no previous therapy of any sort. She was given thirteen doses of P^{32}, totaling 14.1 millicuries, in a period of nine weeks. By the time the course of therapy was completed, she was asymptomatic; enlarged lymph nodes and abdominal masses were no longer palpable. The remission lasted almost eleven months, at the end of which time a second course of therapy again resulted in complete remission. The leucocyte and erythrocyte levels in the peripheral blood were practically unaffected by the treatments.

Kenney and Craver[13] analyzed the results of treatment of eight patients, but five of these had been previously reported.[9] The three new patients all had reticulum cell sarcoma; they were given nineteen doses of P^{32} totaling 16.8 millicuries in a period of three months, ten doses totaling 8.84 millicuries in a period of three weeks, and thirty-five doses totaling 22.8 millicuries in two months time, respectively. The first two patients did not improve, and enlarged lymph nodes remained the same or increased in size. The third individual showed complete regression of skin nodules and enlarged lymph nodes. In all three instances the erythrocyte level dropped significantly following therapy, and the leucocyte and platelet level decreased in the two cases where these determinations were done.

TABLE 17. HEMATOLOGIC DATA ON FIVE PATIENTS WITH LYMPHOSARCOMA, THREE PATIENTS WITH RETICULUM CELL SARCOMA, AND ONE PATIENT WITH GIANT FOLLICULAR LYMPHOBLASTOMA TREATED WITH RADIOACTIVE PHOSPHORUS

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	TOTAL P^{32} THERAPY ADMINISTERED (MC.)	DURATION OF P^{32} THERAPY FROM FIRST INJECTION TO LAST INJECTION (DAYS)	BLOOD COUNTS AT TIME P^{32} THERAPY WAS STARTED (ABOVE) BLOOD COUNTS ONE MONTH AFTER LAST TREATMENT, OR IMMEDIATELY PRIOR TO DEATH (BELOW)							DURATION OF DISEASE FROM FIRST SYMPTOM	
					W.B.C. (PER C.MM.)	R.B.C.[b] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% MYELOID CELLS	% LYMPHOCYTES	% MONOCYTES	TO START OF P^{32} (MO.)	TO DEATH (MO.)
Lymphosarcoma													
L1	46, F	X-R	3.72	12	9,650	4.28	11.0	1,010,000	79	6	15	24	?
					6,950	4.32	11.3	553,000	83	8	9		
L2	51, F	X-R	3.01	129	3,400	4.15	12.2	656,000	70	14	16	15	29
					4,400	3.42	11.2	308,000	54	27	19		
L3	52, M	None	6.29	30	5,200	4.42	14.9	972,000	80	7	13	3	19+
					2,350	4.04	15.0	350,000	72	14	14		
L4	34, M	X-R	6.24	159	9,750	4.30	12.2	1,100,000	78	9	13	12	39+
					5,000	4.29	13.3	526,000	73	17	10		
L5	49, M	X-R	7.83		6,400	4.93	13.0	454,000	57	31	12	17	21½
					2,150	4.50	12.4	45,000	68	19	13		
Reticulum cell sarcoma													
R1	59, M	None	10.86	135	11,400	3.45	11.3	649,000	85	8	7	1	5½
					9,900	3.82 T-5	12.5	266,000	90	3	7		
R2	65, F	X-R	15.08	127	4,150	3.51	10.6	667,000	77	8	15	5	9½
					1,100	1.08	4.2	4,000	40	24	36		
R3	15, F	T	3.00	15	3,250	3.99	14.1	439,000	42	40	11[c]	5	6½
					2,550	3.55	11.9	348,000	6	24	0[d]		
Giant follicular lymphoblastoma													
F1	13, M	X-R	5.9	53	3,200	4.76	15.5	762,000	60	36	4	36?	75+
					1,500	4.31	14.7	232,000	43	48	9		

[a] X-R = X-ray; T = transfusions.

[b] T = Transfusions. The figure indicates the number given.

[c] Seven per cent of the leucocytes in the peripheral blood at this time were reticulum cells.

[d] Seventy per cent of the leucocytes in the peripheral blood at this time were reticulum cells.

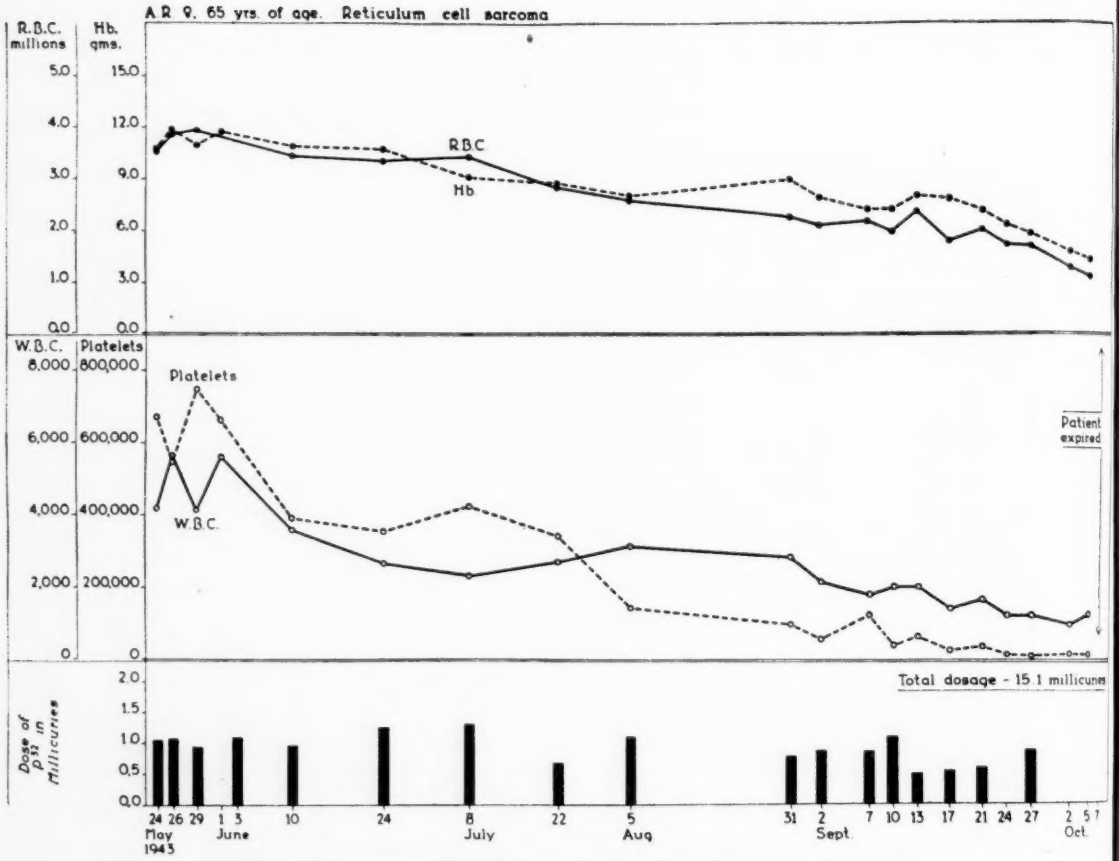

Fig. 16.—Reticulum cell sarcoma. Effect of P^{32} therapy on the blood.

Case Summary.—A. R., a woman 65 years of age, first noted a lump in the right axilla in December, 1942. Because the mass increased rapidly in size, it was removed in January, 1943. Histopathologic examination established the diagnosis of reticulum cell sarcoma. One month later a mass appeared in the right breast; this subsided promptly following x-ray therapy (1,600 roentgens). Early in May, 1943, a mass suddenly developed in the left side of the neck. Following x-ray therapy (1,600 roentgens) this mass decreased in size but did not disappear. During May, 1943, the patient noticed increasing fatigability, dyspnea, and cough.

Physical Examination (May 21, 1943): Blood pressure was 150/95. There was x-ray pigmentation over the neck and anterior thorax. Several rubbery nodes about 1 cm. in diameter were felt in the neck. A firm mass 5 by 6 cm. in size was felt in the right breast. Moist râles were heard at the extreme left base. There was tenderness in the lower abdomen, but no masses were felt, and the liver and spleen were not palpable.

Laboratory Data: Blood counts (May 24, 1943): leucocytes, 4,100 per cubic millimeter; erythrocytes, 3,510,000; hemoglobin, 10.6 Gm.; reticulocytes, 0.4 per cent; platelets, 667,000; differential, normal. Urine was negative. Kahn reaction was negative.

Course: After administration of P^{32} was begun on May 24, 1943, there was little change in patient's weakness and cough. Her appetite remained poor, and she felt drowsy. On May 30, 1943, a small mass about 2 by 3 cm. in size was felt deep in the abdomen just below the umbilicus. Thereafter this mass increased steadily in size until by Aug. 5, 1943,

(Continued on opposite page.)

None of the four patients with lymphosarcoma treated with P^{32} by Warren[15] were helped. Two of them had had no previous therapy and the other two had received x-radiation in the past. No other data were given.

B. *Analysis of the Results Obtained in Treating Five Patients With Lymphosarcoma, Three Patients With Reticulum Cell Sarcoma, and One Patient With Giant Follicular Lymphoblastoma.*—The duration of symptoms in the group of eight patients with lymphosarcoma or reticulum cell sarcoma varied from one to twenty-four months (Table 17). Five of these individuals had had previous x-ray therapy. In Patients L1 and L4 there were no symptoms at the time x-radiation was administered and the only manifestation of disease was enlarged nodes; these disappeared promptly and completely after the treatments. Patient R2 also had no symptoms; a mass in the right breast disappeared completely after her first course of roentgen radiation, but enlarged cervical nodes underwent only partial regression following a subsequent course of x-ray therapy. Marked weakness, the only complaint of Patient L5, was entirely relieved by x-ray. Patient L2 had no symptoms, and the only tumor mass was removed surgically just prior to x-radiation. Thus, therapy cannot be evaluated in this instance.

The total dosage of P^{32} administered varied from 1.1 to 15.1 millicuries, with an average of 7.0 millicuries. In spite of the relatively small dosage employed in most cases, the leucocyte and thrombocyte levels in the blood decreased in all instances, and the erythrocyte level decreased by more than 400,000 cells per cubic millimeter in four cases. The platelet count of Patient R2 dropped to almost zero and she died following massive hemorrhage into the intestines and peritoneum.

Administration of radioactive phosphorus was followed by significant clinical improvement in only two instances. When treatment was started, Patient R1 had pain in the back, frequent night sweats, dyspnea on exertion, profound weakness, and marked anorexia. One month later all of these symptoms had completely disappeared except weakness and anorexia, which had improved. However, in addition to P^{32}, the patient had received five blood transfusions. The clinical remission lasted about three months. Patient L5 had no complaints other than weakness; within two weeks after P^{32} therapy was instituted he felt much stronger, and this symptomatic remission lasted about four months. In all other patients, not only was there no clinical improvement, but symptoms became progressively more severe.

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it measured 10 by 15 cm.; it was rubbery and nodular. The cervical nodes increased in size, and axillary and inguinal nodes appeared. Pitting edema of the back and legs became progressively more marked. By Oct. 6, 1943, the patient was semicomatose, the temperature was 38.5° C., and the abdomen was filled with nodular masses and fluid. She expired five months after therapy with P^{32} had been started.

At post-mortem examination, the patient was found to have reticulum cell sarcoma involving the mediastinal, mesenteric, periaortic, peripancreatic, and iliac lymph nodes; these latter nodes had fused to form tremendous nodular masses. There was sarcomatous involvement of the mucosa of the duodenum and the stomach. Blood was found in the intestine and in the peritoneal cavity (1,500 c.c.).

All of these patients except one had palpable or roentgenologically demonstrable enlargement of lymph nodes. In three instances (Patients R1, R3, and L5) the nodes decreased significantly in size following P^{32} therapy, whereas in the other four they increased progressively in size. The spleen was initially palpable in only two instances (Patients L4 and L5); in Patient L4 progressive enlargement occurred, while in Patient L5 the lower pole of the spleen, which originally measured 23 centimeters below the costal margin in the mid-clavicular line, measured only 12 centimeters two months later. In no case was the regression in size of the lymph nodes or spleen of more than a few months' duration.

Hematologic data and the case report for Patient R2 are shown in Fig. 16.

One patient with giant follicular lymphoblastoma (Brill-Symmer's disease) has been treated. This was a boy, 13 years of age, who had had one slowly enlarging nodule in his neck for three years, and several other cervical nodes for about six months. His mother insisted he had tired easily for two to three years, but the boy denied this. There were no abnormal physical signs other than moderate enlargement of the cervical and axillary nodes. The blood counts were normal in all respects. Biopsy of a cervical node revealed the typical microscopic appearance of giant follicular lymphoblastoma. During a period of seven months he was given thirteen injections of P^{32} totaling 5.91 millicuries (Table 17). At the end of this time there were no palpable nodes, and the patient played on his school basketball team. During the course of treatment, the leucocyte level dropped as low as 1,500 and did not return to normal until a year later; the other cellular elements in the blood were unaffected. About eighteen months after completion of the course of treatment several palpable cervical and axillary nodes reappeared. One of the cervical nodes was biopsied; the sections did not show the changes of giant follicular lymphoblastoma but only scattered areas of fibrosis. The patient remains asymptomatic, and the blood counts are all normal two years after the first treatment.

C. *Summary.*—There are several reports in the literature which suggest that P^{32} may be of value in the treatment of lymphosarcoma, reticulum cell sarcoma, and giant follicular lymphoblastoma. Twenty-one patients have been treated and followed for a sufficient length of time so that it was possible to draw at least tentative conclusions regarding the effect of the therapy; in six patients complete regression of all symptoms and signs of disease was observed, in four patients partial regression occurred, and in eleven there was no detectable improvement. In no instances had the remission lasted more than eleven months at the time of the report. Radioactive phosphorus therapy was considered a failure in all of the patients with lymphosarcoma and reticulum cell sarcoma treated at the Mallinckrodt Institute of Radiology. Relief of symptoms occurred in only two patients, in one of whom five transfusions probably accounted for the improvement, and, in the other, increase in strength was the only evidence of benefit. Enlarged lymph nodes decreased in size in three instances, and an enlarged spleen became smaller in one instance. The cellular elements of the blood decreased significantly in number following treatment. In conclusion, it is felt that the unfavorable influence of P^{32} therapy on the bone mar-

row more than offsets the occasional clinical benefit which may occur following such treatment. It is our impression that x-radiation is a more effective and less dangerous form of therapy than P^{32} for this group of diseases.

IX. MULTIPLE MYELOMA

Under this heading are included a few cases in which plasma cells were found in the peripheral blood and bone marrow in large numbers. Some authors have labeled these as plasma cell leukemia and probably would have made a similar diagnosis in Patient 4 in our series (Table 18). This man had typical multiple myeloma with numerous characteristic x-ray lesions in the skull, pelvis, humerus (pathologic fracture also present), and left femur; Bence-Jones proteinuria was present; 28 per cent of the cells in the bone marrow were plasma cells; and a biopsy of the lesion of the left humerus taken at the time of open reduction of the fracture showed multiple myeloma. The diagnosis was confirmed by post-mortem examination. When this patient was first observed there were no plasma cells in the peripheral blood, but during the course of P^{32} therapy they appeared in the blood in increasing numbers and finally accounted for 20 per cent of all the leucocytes at the time of death. All cases will be discussed together without differentiation as to whether myeloma cells were found in the peripheral blood.

A. *Review of the Literature.*—There are four reports[5, 10, 12, 15] which describe the results of therapy with radioactive phosphorus in seventeen patients with multiple myeloma or plasma cell leukemia. Case reports are given for only three of these individuals.

Erf and associates[5] treated a man, 59 years of age, who had weakness and aching pains of two weeks' duration. There were many plasma cells in the blood and bone marrow and large amounts of Bence-Jones protein in the urine. The diagnosis of acute plasmacytoid leukemia was made, and 14.9 millicuries of P^{32} were administered orally in nineteen days. Death occurred two months after treatment was started; post-mortem examination revealed plasma cell infiltration of most organs including the bone marrow, kidneys, and submucosa of the gastrointestinal tract.

Fitz-Hugh and Hodes[10] state that the one patient with multiple myeloma whom they treated with P^{32} was not benefited by the drug. No data were given.

Eleven patients were treated by Low-Beer and co-workers.[12] The authors stated that at the time of the report the response of these individuals to P^{32} therapy had not been uniform. In some patients there had been marked relief of pain and a restoration to almost normal activity. However, no appreciable change in the roentgenographic appearance of the lesions had occurred. A case report was given for one patient, a man 49 years of age, who had been found to have multiple myeloma fifteen months earlier. Previous x-ray therapy to the spine and left leg had afforded partial relief from pain. During a period of five months, 5.0 millicuries of P^{32} had been given orally and 13.8 millicuries intravenously. The patient's physical activity had been very limited but several months after the first dose of P^{32} he became able to walk and drive his automobile. Without giving any specific data, the authors state that, "There was no adverse change in the blood levels during or after treatment."

Three patients with "plasmacytoma" and one with plasma cell leukemia were treated with P^{32} by Warren.[15] Two of the patients with plasmacytoma were said to have been helped by the treatment. A case report was given for one patient, a woman 66 years of age, who had had symptoms for about one year. X-ray studies showed lesions throughout the whole skeleton; 6 per cent of the leucocytes in the peripheral blood were plasma cells; and the sternal marrow contained masses of plasma cells. Between Nov. 12, 1941, and April 14, 1942,

TABLE 18. HEMATOLOGIC DATA ON EIGHT PATIENTS WITH MULTIPLE MYELOMA TREATED WITH RADIOACTIVE PHOSPHORUS

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	DATA UPON WHICH DIAGNOSIS WAS BASED[b]	TOTAL P^{32} THERAPY ADMINISTERED (MC.)	DURATION OF P^{32} THERAPY FROM FIRST INJECTION TO LAST INJECTION (DAYS)	BLOOD COUNTS AT TIME P^{32} THERAPY WAS STARTED (ABOVE) BLOOD COUNTS ONE MONTH AFTER LAST TREATMENT, OR IMMEDIATELY PRIOR TO DEATH (BELOW)								DURATION OF DISEASE FROM FIRST SYMPTOM	
						W.B.C. (PER C.MM.)	R.B.C.[c] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% MYELOID CELLS	% LYMPHOCYTES	% MONOCYTES	% PLASMA CELLS	TO START OF P^{32} (MO.)	TO DEATH (MO.)
1	47, F	X-R	B, M(7%), X, P	16.35	1096	4,550	2.64	11.2	808,000	74	21	5	0	36	62
						4,300	2.95 T-12	9.7	348,000	77	12	11	0		
2	66, M	None	M(14%), G, A	5.88	35	7,200	4.04	12.9	582,000	71	23	6	0	12?	20
						3,500	3.67	12.7	338,000	66	26	8	0		
3	62, F	None	B, M(90%), X, A	8.66	52	11,000	2.29	8.0	550,000	83	12	5	0	12	14
						1,500	1.33 T-3	4.5	54,000	90	0	10	0		
4	49, M	None	B, M(28%), X, P, A	5.00	14	7,500	4.19	11.6	1,307,000	66	22	11	1	9	10
						16,200	3.90	11.8	1,518,000	72	5	3	20		
5	53, F	None	B, M(46%), P, G, A	10.91	110	10,050	2.48	8.9	600,000	46	31	15	8	36	41
						1,900	2.02 T-3	5.7	12,000	30	56	6	8		
6	69, F	LE	M(48%), X, P, G	4.89	21	4,000	1.80	6.3	640,000	65	26	7	2	36?	38
						2,400	1.79 T-3	6.3	100,000	70	24	4	2		
7	44, M	None	M(99%), X, P	16.75	253	10,800	4.48	13.1	910,000	77	16	7	0	12	21+
						9,500	3.34 T-3	12.4	354,000	78	15	7	0		
8	58, M	None	M(83%), X, G	8.63	145	6,400	2.00	8.1	224,000	36	52	10	2	9	17+
						3,350	2.50 T-5	10.1	20,000	32	59	8	1		

[a]X-R = X-ray; LE = liver extract.

[b]Each letter listed indicates that the corresponding test or examination was positive, or that the results were compatible with the diagnosis of multiple myeloma. B = Biopsy of tumor mass; M = percentage of plasma cells in sternal marrow; X = x-ray films of skeleton including skull; P = Bence-Jones proteinuria; G = elevated serum globulin; A = autopsy.

[c]T = Transfusions. The figures indicate the number given.

she received 12.1 millicuries of P^{32} intravenously. Bone pain disappeared promptly after treatment was started, and five months later x-ray examination showed some recalcification of the osteolytic foci. The erythrocyte and leucocyte levels were both within normal limits at this time. Between Sept. 24, 1942, and July 31, 1943, she received an additional 9.67 millicuries of P^{32}. Her clinical course was complicated by the development of a duodenal ulcer. She was transfused repeatedly. In November, 1943, she developed lobar pneumonia from which she died; the leucocyte count at this time was 1,550, and the erythrocyte count was 1,630,000. The author felt that this patient showed appreciable improvement lasting from November, 1941, to February, 1943.

B. *Analysis of the Results Obtained in Treating Eight Patients With Multiple Myeloma With Radioactive Phosphorus.*—The diagnosis of multiple myeloma in all eight patients included in this study was accepted only after myeloma cells were found in the bone marrow. Four of the patients had tumor masses which were biospied and which showed the characteristic histopathologic changes of multiple myeloma. Supporting evidence for the diagnosis was obtained from chemical and roentgenologic studies (see column 4 of Table 18). Necropsy has been performed on four of the patients.

The duration of symptoms at the time therapy with P^{32} was started varied from nine to thirty-six months, with an average of twenty months. Only one patient (Patient 1) had received previous radiation therapy; she had had severe pain in the spine which was markedly relieved by x-radiation.

The total dosage of P^{32} administered ranged from 5.0 to 16.7 millicuries, with an average of 9.6 millicuries. The leucocyte level decreased to below 4,000 cells per cubic millimeter in five instances and below 2,000 in two instances; the thrombocyte level fell to 100,000 per cubic millimeter or less in four patients (Table 18). The leucocyte count in Patient 3 dropped as low as 550 cells per cubic millimeter three weeks prior to death and fluctuated thereafter between 800 and 2,100; bilateral pneumonia with septicemia due to hemolytic *Staphylococcus aureus* was the immediate cause of death. In Patient 5, the leucocyte level ranged between 1,400 and 2,300 cells per cubic millimeter during the last three weeks of life. She developed a meningitis and a bacteremia (pneumococcus type 29); this infection was not controlled even with intensive therapy with penicillin and sulfadiazine. Platelets virtually disappeared from the blood during the last two months of this patient's life; she developed extensive petechiae and ecchymoses and a terminal subarachnoid hemorrhage. There can be little doubt but that depression of leucocyte and platelet formation in the bone marrow by the P^{32} was the underlying factor responsible for death. The effect of radioactive phosphorus on erythrocytogenesis in these patients could not be estimated accurately because all but two of them received repeated blood transfusions.

Treatment with radioactive phosphorus was followed by clinical improvement in only two instances. When therapy was started, Patient 8 (Table 18) had no symptoms except dyspnea and marked weakness. One month later he felt much stronger and dyspnea had disappeared. It was felt that this improvement was due primarily to five blood transfusions which he had received during the course of P^{32} therapy. The other patient who improved (Patient 6) also received transfusions (three) which might have accounted for increase in

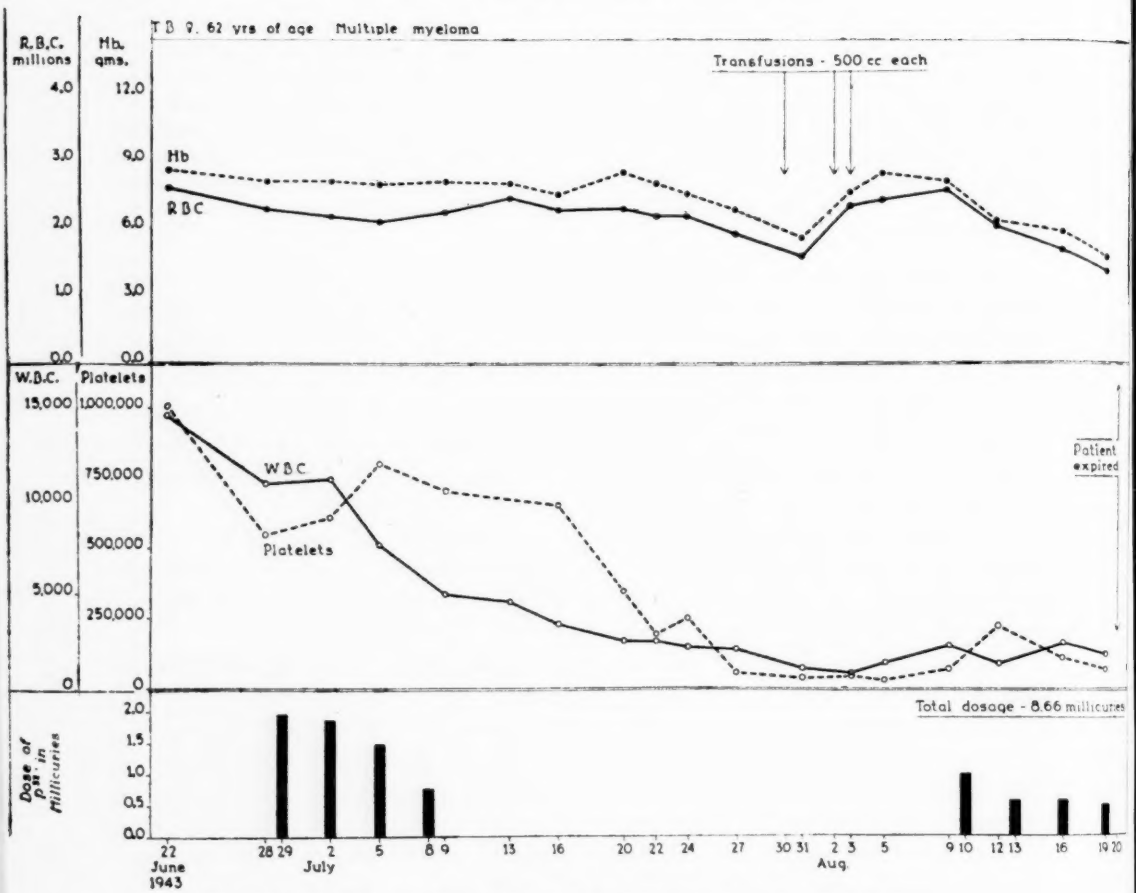

Fig. 17.—Multiple myeloma. Hematologic changes following P^{32} therapy.

Case Summary.—T. B., a married woman, 62 years of age, had an attack of pain in her shoulders and spine lasting two weeks in June, 1942. She then felt perfectly well until February, 1943, when her legs became weak; this was followed first by numbness and then by paralysis of the legs. The paralysis gradually ascended, and by April, 1943, she was totally paralyzed from the waist down. During June she developed blurring of vision, diplopia, and a coarse tremor of the hands.

Physical Examination (June 28, 1943): Blood pressure was 200/100. The patient was moderately obese. Her back was stiff, and there was tenderness to percussion over the upper dorsal vertebrae. The bladder was distended to the umbilicus. Complete flaccid paralysis of both lower extremities was present together with some weakness and spasticity of the upper extremities. There was complete sensory loss below the level of the sixth dorsal segment. Abdominal, knee jerk, and ankle jerk reflexes were absent bilaterally.

Laboratory Data: Urinalysis was negative, no Bence-Jones protein was present. The Kahn reaction was negative. Total plasma proteins were 6.2 Gm. per cent, with albumin 3.1 Gm., and globulin 3.1 Gm. Basal metabolic rate was +15 per cent. Blood counts (June 22, 1943): leucocytes, 14,650; erythrocytes, 2,580,000; hemoglobin, 8.5 Gm.; reticulocytes, 2.8 per cent; platelets, 1,050,000; differential, normal. Sternal bone marrow examination (June 29,

(Continued on opposite page.)

strength. However, in addition, she also experienced marked relief of pain. Prior to P^{32} therapy she had severe pain in the back and flanks, hands, shoulders, and the right knee. One month later these pains had entirely disappeared. The patient said she had had arthritis for thirty years, and an x-ray of the knee showed osteoarthritic changes. X-rays of the spine showed compression fractures of the ninth and tenth dorsal vertebrae. Thus, it is difficult to evaluate how much, if any, of the relief of pain could be attributed to the therapy with radioactive phosphorus.

No improvement in physical signs occurred in any of these individuals. In one instance (Patient 1) it was felt that x-rays of the skeleton taken after treatment when compared with earlier films showed some evidence of repair proceeding in the bone lesions as evidenced by stationary size, a tendency to demineralization, and sharper outline of the defects by deposits of dense bone in their margins.

One patient (Patient 4) developed uremia. On admission to the hospital his plasma nonprotein nitrogen level was 31 milligrams per cent, and the uric acid level was 7.3 milligrams per cent. Two weeks later, after having received 5.0 millicuries of P^{32}, he was disoriented; the nonprotein nitrogen value had risen to 180 milligrams per cent, and the uric acid to 16.6 milligrams per cent. It was felt that rapid necrosis of tumor cells as a result of radioactive phosphorus administration might have contributed to the azotemia. At post-mortem examination, the tubules of both kidneys were found to be blocked with deposits of urates.

Hematologic data and the case report for Patient 3 are shown in Fig. 17.

C. *Summary.*—Radioactive phosphorus has not proved to be a valuable therapeutic agent for the treatment of multiple myeloma. In no case has there been any convincing objective evidence of improvement following treatment. Low-Beer and associates[12] and Warren[15] reported relief of pain in a few patients; Patient 6 in our series experienced considerable relief of pain, but the causal relationship of this improvement to P^{32} was obscure. It is our impression that radiophosphorus therapy significantly shortened the duration of life in two patients by producing severe leucopenia and thrombocytopenia.

1943) showed: plasma cells, 90 per cent; myeloid cells, 8 per cent; primitive cells, 1.5 per cent; and nucleated red blood cells, 0.5 per cent.

X-ray films revealed lesions typical of multiple myeloma in the skull, ribs, and spine.

Course: Three weeks after P^{32} therapy was started the patient developed cellulitis of the buttocks; this progressed in spite of sulfadiazine therapy. A week later a purpuric skin lesion appeared on the shoulders. Terminally, she developed bilateral bronchopneumonia, and a blood culture was positive for hemolytic *Staphylococcus aureus.* She expired fifty-three days after treatment was started.

A second sternal marrow aspiration performed on the thirty-sixth day of P^{32} therapy revealed a hypercellular marrow; 93 per cent of the cells were plasma cells, 4 per cent were nucleated red blood cells; no megakaryocytes were seen.

Post-mortem examination revealed plasma cell myeloma involving the marrow of all bones examined (vertebrae, ribs, and sternum), collapse of the sixth thoracic vertebra, multiple abscesses in the myocardium, kidneys, and vertebrae, bronchopneumonia with abscess formation of all lobes of the lungs, and hemorrhagic cystitis of the urinary bladder.

TABLE 19. HEMATOLOGIC DATA ON EIGHT PATIENTS WITH VARIOUS DISEASES TREATED WITH RADIOACTIVE PHOSPHORUS

PATIENT	AGE AND SEX	PREVIOUS TREATMENT[a]	DIAGNOSIS	TOTAL P^{32} THERAPY ADMINISTERED (MC.)	DURATION OF P^{32} THERAPY FROM FIRST INJECTION TO LAST INJECTION (DAYS)	BLOOD COUNTS AT TIME P^{32} THERAPY WAS STARTED (ABOVE) BLOOD COUNTS ONE MONTH AFTER LAST TREATMENT, OR IMMEDIATELY PRIOR TO DEATH (BELOW)							DURATION OF DISEASE FROM FIRST SYMPTOM	
						W.B.C. (PER C.MM.)	R.B.C.[b] (MILLIONS PER C.MM.)	HG (GM. PER 100 C.C.)	PLATELETS (PER C.MM.)	% MYELOID CELLS	% LYMPHOCYTES	% MONOCYTES	TO START OF P^{32} (MO.)	TO DEATH (MO.)
1	20, M	Sur	Ewing's tumor	5.3	8	15,050	4.70	14.7	1,130,000	64	16	20	3	?
						10,600	4.46	14.1	1,600,000	68	10	22		
2	9, F	None	Ewing's tumor	3.1	18	7,200	3.79	9.2	857,000	79	15	6	2	?
						5,700	3.48	9.2	835,000	76	11	13		
3	17, M	X-R	Ewing's tumor	11.5	53	10,000	4.59	10.7	421,000	70	15	15	2	5
						1,950	0.78	2.2	65,000	78	11	11		
4	18, F	None	Malignant melanoma	7.7	26	7,850	2.84	8.0	485,000	85	4	11	4	5
						3,100	1.45	3.8	190,000	74	14	12		
5	52, M	None	Anaplastic carcinoma	12.9	288	5,950	4.10	11.8	1,390,000	64	28	8	6	28+
						3,250	4.13	11.7	326,000	53	26	21		
6	55, M	FT	Mycosis fungoides	18.4	549	7,100	4.57	15.4	1,080,000	87	8	5	18	54+
						4,570	3.75	12.0	367,000	84	8	8		
7	38, F	X-R, FT	Mycosis fungoides	15.0	111	8,600	4.45	15.0	1,110,000	78	7	15	72	83+
						6,550	4.23	10.7	711,000	77	13	10		
8	1, M	None	Xanthomatosis	0.33	4	6,050	3.10	7.9	127,000	44	48	8	7	7⅓
						11,150	4.75 T-1	13.8	38,000	68	30	2		

[a]Sur = Surgery; X-R = x-ray; FT = fever therapy.
[b]T = Transfusions. The figure indicates the number given.

X. MISCELLANEOUS DISEASES

A. *Review of the Literature.*—Eight patients with osteogenic sarcoma, one with metastasizing hemangioma, one with a lymphoepithelioma of the tonsil with widespread metastasis, one with melanoma with numerous cutaneous metastases, and one with extensive mycosis fungoides were treated with P^{32} by Kenney.[9] All but one of the patients with osteogenic sarcoma were still being treated at the time of the report, and no estimate of the end results of the therapy was possible. One patient who had refused amputation, and whose primary tumor had received a 9,100 "r" tumor dose of 1,000 kv. x-ray at 70 centimeters T.S.D., was given 20 millicuries of radioactive phosphorus over a period of three months. The extensive metastases continued to grow during the period of therapy with P^{32}. The patient with metastasizing hemangioma received a total dose of 150 microcuries per kilogram of body weight in eighteen days; this was divided into small daily doses. No regression was observed during life, nor were any changes that could be attributed to the P^{32} noted in the tumor tissue at autopsy twenty days after the commencement of therapy. The patient with metastases from a lymphoepithelioma of the tonsil was given 350 microcuries per kilogram of body weight in thirty-four days. No regression of the lesions was noted at any time. The patient with cutaneous metastases from a melanoma received 350 microcuries per kilogram of body weight in divided doses in forty days. The lesions increased steadily in size during and after the administration of P^{32}. The patient with mycosis fungoides was given 200 microcuries per kilogram of body weight in nineteen days. There was some regression of the skin lesions, but the individual left the hospital before the course of therapy was considered complete and he was not seen again.

Fitz-Hugh and Hodes[10] administered P^{32} to two individuals with extensive gall bladder carcinoma and one with disseminated carcinoma of the breast. None of these patients was benefited by treatment with the isotope. The dosage employed was not stated.

B. *Analysis of the Results Obtained in Treating Three Patients With Ewing's Sarcoma, One With Malignant Melanoma, One With Anaplastic Carcinoma (Primary Site Undetermined), Two With Mycosis Fungoides, and One With Xanthomatosis.*—The diagnosis in all eight of these patients was confirmed by biopsy. Two of the patients had been treated previously with x-radiation. One individual (Patient 7, Table 19) had had mycosis fungoides for six years; at various times he had received fever therapy and several courses of x-ray, neither of which forms of treatment had had any significant effect on the course of the disease. Patient 3, a 17-year-old boy with Ewing's sarcoma, had just completed a course of x-ray therapy (2,400 roentgens to the primary tumor which arose from a rib on the right side); this considerably lessened the pain he was having in the right chest.

The total dosage of P^{32} administered to each of these individuals is recorded in Table 19. In two instances the dosage was very small. Patient 2, a girl, 9 years of age, with Ewing's tumor arising from the right ileum, was given 3.1 millicuries of P^{32} during eighteen days. Throughout this time she suffered from severe pain in the right hip and right leg; as this pain was becoming steadily worse, therapy with P^{32} was discontinued and x-radiation started. She was given 400 roentgens daily for twelve days, at the end of which time pain was somewhat less intense. She then returned to her home in another city, and no observations regarding the effect of the x-radiation on the size of the tumor are available. Patient 8, a male infant 12 months of age with xanthomatosis, weighed 15 pounds. He was given only 0.33 millicuries of radioactive phosphorus in three injections during a period of four days. Death oc-

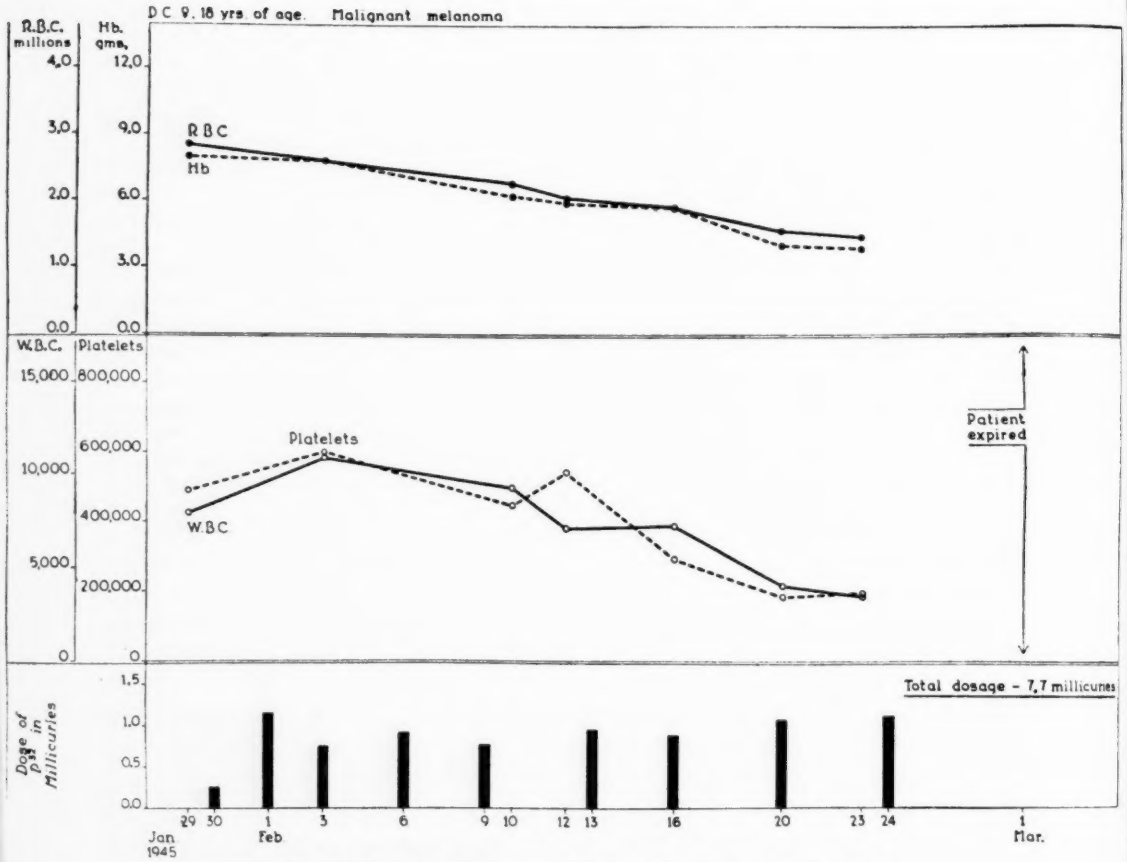

Fig. 18.—Malignant melanoma. Effect of P^{32} therapy on the blood.

Case Summary.—D. C., a young woman 18 years of age, had had numerous moles scattered over her body since childhood. In January, 1944, a mole which was increasing in size was removed from the left arm; at the same time large moles were removed from the back and from the left axilla. In August, 1944, she noted a lump in the left axilla; it enlarged rapidly. In November, 1944, this mass was biopsied and was found to be a malignant melanoma. A month later she was given x-ray therapy totaling 2,300 roentgens to the left axilla. About December 28 she developed severe low back pain, weakness, tinnitus, anorexia, weight loss, and fever.

Physical Examination: The temperature was 39° C. There was a striking pallor of the skin and mucous membranes. A stony hard mass measuring 5 by 5 cm. was present in the left axilla, and five stony hard, freely movable subcutaneous nodules were palpable in the abdominal wall. Breath sounds were distant, and the percussion note was hyporesonant bilaterally.

Laboratory Data: Blood counts (Jan. 29, 1945): leucocytes, 7,850 per cubic millimeter; erythrocytes, 2,840,000; hemoglobin, 8.0 Gm.; reticulocytes, 2.0 per cent; platelets, 486,000; differential, normal. Sternal bone marrow (Jan. 30, 1945): the marrow was very dense and hypercellular; 60 per cent of the cells in the marrow were malignant sarcoma cells. Urine was negative. Kahn reaction was negative. Serum proteins, normal.

(Continued on opposite page.)

curred nine days after treatment was started. It is recognized that in both of these patients the dosage employed was inadequate to permit evaluation of the treatment.

None of the patients considered in this section was benefited either subjectively or objectively by P^{32} therapy. In no instance did a palpable or roentgenologically demonstrable tumor mass decrease in size during or following administration of the isotope. Patient 3 (Ewing's tumor) was given 11.5 millicuries of P^{32} in fifty-three days; this dose was sufficient to produce profound anemia, leucopenia, and thrombocytopenia. In spite of the fact that therapy was pushed beyond the point of tolerance, several visible tumor masses arising from the skull bones increased at least 50 per cent in size during the course of isotope administration. Patient 4, a girl 18 years of age with melanosarcoma, was given 7.7 millicuries in twenty-six days, during which time she also developed anemia, leucopenia, and thrombocytopenia. Not only did the axillary and subcutaneous metastases present at the time P^{32} administration was started increase in size, but numerous new tumor masses appeared. Patient 5 had an anaplastic carcinoma involving the right nasopharynx and adjacent structures; there was marked exophthalmus on the right and extensive involvement of the frontal bone bilaterally. Even though 9.0 millicuries were administered in sixty-six days, all visible and roentgenologically demonstrable masses became larger; x-ray therapy (3,400 roentgens in thirteen days) was then administered, following which there was some decrease in size. A month later P^{32} therapy was resumed; this time he was given 3.9 millicuries within a period of six months. During this time, a stony hard mass appeared in the right side of the neck which increased in size until it measured 7 by 7 centimeters. A month after P^{32} was discontinued, he was given x-radiation, 300 roentgens daily for four days, to the lesion in the neck. One month later the mass in the neck had decreased 75 per cent in size. Both patients with mycosis fungoides (Patients 6 and 7) showed slow progression of their skin lesions while they were under treatment with P^{32}. Following discontinuance of P^{32} administration, Patient 6 was given weekly x-ray treatments by his dermatologist for one year. Approximately 1,600 roentgens were administered to most areas of the body; local areas of tumefaction received larger dosages than this. The local tumors decreased in size at first, but during the latter part of the year, the lesions again showed progression. The subsequent course of Patient 7 is not known.

Hematologic data and the case report for the patient with malignant melanoma (Patient 4) are given in Fig. 18.

Course: Following onset of therapy with P^{32} the pain in the back diminished. However, the patient's appetite steadily worsened, and weight loss and weakness rapidly progressed. High swinging fever persisted. By Feb. 15, 1945, she was semistuporous, and respirations were Cheyne-Stokes in character. During the first two weeks of therapy the mass in the left axilla decreased very slightly in size; thereafter both this mass and the subcutaneous nodules slowly enlarged. The patient died thirty-one days after the first dose of P^{32} had been given.

At post-mortem examination, metastases of malignant melanoma were found in the skin, the lungs, the liver, the spleen, the ovaries, the kidneys, the adrenals, the pancreas, the intestinal wall, the heart, the dura, the leptomeninges, and in lymph nodes throughout the body.

C. *Summary.*—Only a few patients with any of the diseases discussed in this section have been treated with P^{32}. However, the data on these individuals suggest that radioactive phosphorus, in the form in which it has been administered, is not a satisfactory therapeutic agent for the treatment of lymphoepithelioma, malignant melanoma, carcinoma of the gall bladder, carcinoma of the breast, Ewing's tumor, mycosis fungoides, or xanthomatosis.

XI. GENERAL SUMMARY AND CONCLUSIONS

Each section of this paper has been sumarized independently, and only final conclusions will be recorded here.

Radioactive phosphorus emits beta-rays capable of producing a profound radiation effect on tissues. The therapeutic use of this substance has certain advantages compared with x-radiation: it is selectively concentrated in organs with a high phosphorus content such as bone and in tissues the cells of which are multiplying rapidly; it is easy to administer, and therapeutic does never give rise to radiation sickness. The half-life of P^{32} (14.3 days) permits steady radiation of tissues for several weeks, yet is short enough so that the destructive effect on tissues can be controlled.

The literature on radioactive phosphorus therapy has been analyzed, and the results obtained in the treatment of 155 patients at the Mallinckrodt Institute of Radiology have been presented. The available data appear to us to justify the following conclusions:

1. Radioactive phosphorus is probably the best therapeutic agent available at the present time for polycythemia vera. Complete hematologic and almost complete symptomatic remissions can be produced with P^{32} in the vast majority of patients, and remission from a single course of treatment may last for from six months to several years or longer. It is not possible at present to evaluate the effect of radioactive phosphorus therapy on the duration of life of these individuals.

2. Therapy with P^{32} has very little effect on the clinical course of patients with acute or subacute myelogenous leukemia but produces at least as complete clinical and hematologic remissions as x-radiation in the chronic form of the disease. Freedom from radiation sickness is a practical advantage which patients who have had previous x-ray therapy appreciate. The duration of life from the first symptom until death of the patients treated at the Mallinckrodt Institute of Radiology suggests that P^{32} therapy prolongs life to approximately the same extent as does x-ray therapy (about six months).

3. In the great majority of patients, the clinical course of acute lymphatic leukemia is not favorably influenced by P^{32} therapy. In a few patients clinical improvement has been observed, and one patient had almost complete hematologic and symptomatic remission of five and one-half months' duration. However, equally dramatic spontaneous remissions of acute lymphatic leukemia have been observed in our clinic and elsewhere. In the treatment of chronic lymphatic leukemia, P^{32} is probably as satisfactory as, but no better than, roentgen radiation.

4. Radioactive phosphorus is of no value in the treatment of monocytic leukemia.

5. Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and multiple myeloma do not respond as favorably to P^{32} as they do to x-radiation.

6. From observations on the few patients who have been treated, there is no reason to believe that P^{32} is a satisfactory therapeutic agent for the treatment of lymphoepithelioma, malignant melanoma, carcinoma of the gall bladder, carcinoma of the breast, Ewing's tumor, mycosis fungoides, or xanthomatosis.

7. In patients with all the various types of diseases which have been treated, P^{32} has been shown to have a profound effect on the bone marrow, and severe leucopenia, thrombocytopenia, and anemia may occur as complications of the therapy. There is wide variation in the dosage of radioactive phosphorus required to produce these complications in different individuals. When more than one of the cellular elements of the marrow were depressed in the same individual, the cytologic changes in the peripheral blood usually occurred in the following order: the leucocyte level decreased first, the thrombocyte level second, and the erythrocyte level was affected last.

8. In patients with any type of chronic leukemia, roentgen radiation is more effective in some cases than P^{32} in bringing about a rapid reduction in the size of the spleen or lymph nodes. Therefore, x-ray should be employed whenever the prompt reduction of nodes or the spleen is necessary to relieve symptoms or remove pressure on some vital organ. X-ray may be used to supplement radioactive phosphorus therapy for this purpose.

9. Detailed suggestions as to methods of administering P^{32} and the proper dosage range for the treatment of patients with polycythemia vera, chronic myelogenous leukemia, and chronic lymphatic leukemia have been recorded. Therapy must be individualized to a high degree, and the final total dosage for any patient must be determined on the basis of repeated blood and bone marrow studies and clinical observations.

10. At present, several different methods are employed in different laboratories for the assay of the activity of solutions of radioactive phosphorus salts. These methods have been discussed by Dr. Martin D. Kamen in the Appendix. When the same sample of radioactive di-sodium acid phosphate solution was assayed in several laboratories, widely divergent results were obtained. This fact must be kept in mind in calculating dosage. An attempt is being made to correct this discrepancy.

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(APPENDIX FOLLOWS)

APPENDIX

APPENDIX TABLE 1. CLINICAL RESULTS

PATIENT	BEFORE TREATMENT WITH P^{32}		AFTER P^{32}
	SYMPTOMS	PHYSICAL SIGNS DUE TO POLYCYTHEMIA	SYMPTOMS
1	**Fatigability, headaches, itching, dizzy spells, vertigo, numbness and aching of extremities (7 yr.)**	Skin and mucous membranes dark red; cyanosis; conjunctivae red; spleen 6 cm. below costal margin	All symptoms persisted but became less severe and less frequent
2	**Fatigability, headaches**	Skin and mucous membranes red; spleen 3 cm. below costal margin	All symptoms improved greatly for 18 mo., then became worse; continued to have headaches
3	Itching and lacrimation (7 yr.), fatigability (1 yr.)	Spleen 12 cm. below costal margin	Each course of P^{32} therapy completely relieved all symptoms until R.B.C. count again rose
4	Fatigability, headaches, aching pains in legs, dizziness, dyspnea, depression, forgetfulness, nosebleeds, blurring of vision	Skin and mucous membranes dark red; petechiae and ecchymoses; conjunctivae red; apical systolic murmur; spleen 4 cm. below costal margin	All symptoms disappeared except headaches, dizziness, and dyspnea, which lessened in frequency and severity
5	Headaches, tinnitus, vertigo, fatigability (16 yr.); 6 epileptiform convulsions (11 yr.); itching, bloating, belching, aching in extremities (3 yr.)	Skin and mucous membranes intensely red, cyanotic; fundi—venules distended; spleen 3 cm. below costal margin; liver 5 cm. below costal margin	No tinnitus, vertigo, fatigability, or aching in extremities; occasional mild headaches, itching, and bloating; convulsions as before
6	Headaches, numbness and tingling in hands and anorexia; 8 mo. prior to P^{32} therapy had sudden left hemiparesis which gradually cleared up	Skin and mucous membranes somewhat red; lips cyanotic; spleen 4 cm. below costal margin	No headaches or numbness and tingling in hands; appetite much better
7	Severe headaches with nausea and vomiting, transient episodes of unconsciousness, burning of eyes and lacrimation	None except spleen is 3 cm. below costal margin. B.P., 150/80	All symptoms completely relieved; still feels well 33 mo. after treatment
8	Numbness and pain in hands and feet, precordial pain, pain in both shoulders, fatigability, drowsiness	Skin and mucous membranes dark red, cyanotic; B.P., 185/115; heart enlarged; spleen 18 cm. below costal margin; liver 14 cm. below costal margin	Fatigability, drowsiness greatly improved; precordial pain and pain in shoulders and hands unimproved
9	Left hemiplegia (6 mo.), headaches, blurring of vision, fatigability, loss of memory, convulsion (4 mo. previously)	Skin and mucous membranes dark red, cyanotic; B.P., 150/120; apical systolic murmur; spleen 3 cm. below costal margin, left hemiplegia	Fatigability, headaches, vision and memory somewhat improved; no change in hemiplegia; continued to have convulsions
10	Severe headaches, fatigability, aching and tingling of extremities, lower abdominal pain	Spleen 4 cm. below costal margin	Headaches improved; fatigability no better; patient miserable because of pains "all over body," weakness, dyspnea, and palpitation
11	Headaches, dizzy spells, severe itching, substernal pain, aching in hips and thighs, moderate fatigability	Skin and mucous membranes red; ecchymoses on thighs; B.P., 155/100; spleen tip palpable just at costal margin	Itching and substernal pain disappeared entirely; other symptoms lessened in severity
12	Sensation as though head were twice normal size, headaches, dizziness, nervousness, blurring of vision	Skin and mucous membranes brilliantly red; nails and lips cyanotic; engorgement of venules (fundi); B.P. 150/120; spleen 12 cm. below costal margin; liver 4 cm. below costal margin	All symptoms completely relieved except for nervousness and an occasional slight dizzy spell

IN THIRTY PATIENTS WITH POLYCYTHEMIA

THERAPY	PHYSICAL SIGNS	REMARKS
	No abnormal findings; spleen not palpable	Course complicated by marked nervousness, severe menopausal symptoms, and marital strife
	No abnormal findings except barely palpable spleen for 15 mo.; many findings thereafter	Two years after onset of therapy, patient developed progressive leucocytosis, anemia, thrombocytopenia; died with "myelogenous leukemia"
	Spleen 1-4 cm. below costal margin	Phlebotomies (12 liters in 4 yr.) prior to P^{32} therapy only partially relieved symptoms
	Color normal; no petechiae or ecchymoses; conjunctivae clear; no murmurs; spleen not palpable	Patient 60 yr. old; has had hypertension (160-180/100-110) for many years; fundi show sclerotic changes, and EKG showed "myocardial damage"
	Color normal; no distention of venules (fundi); spleen barely palpable on deep inspiration; liver not palpable	Patient 42 yr. old; electroencephlogram did not show changes suggestive of epilepsy; much greater relief from P^{32} than from phenylhydrazine taken previously
	Color normal; spleen not palpable	Patient also had fine tremor (? postencephalitic) and hypogonadism; left town 6 mo. after treatment; not seen thereafter
	No abnormal findings; spleen not palpable; B.P., 125-135/70-80	Treated for 4 yr. prior to P^{32} therapy by repeated venesection (about 500 c.c. every eight weeks) with partial relief of symptoms
	Color pale; B.P., 150-160/80-110; heart unchanged; spleen 5 cm. below costal margin; liver 10 cm. below costal margin	EKG showed right bundle branch block; x-ray of spine showed marked hypertrophic spurring of cervical vertebrae
	Color normal; B.P., 110-120/70-95; no murmurs; spleen not palpable; hemiplegia unchanged	Neurologic signs suggested thrombosis with encephalomalacia involving the right pyramidal system as cause of the hemiplegia
	Tip of spleen just felt at costal margin on inspiration	Previously treated with Fowler's solution, phenylhydrazine, and phlebotomies with indifferent results; diagnosed by psychiatrist as having severe psychoneurosis
	No abnormal findings except slight hypertension; B.P., 145-160/80-100	During last year patient has complained of numbness of feet and pain and stiffness in hands; x-rays negative
	Color normal; no cyanosis or engorgement of venules; B.P., 140/100; spleen 7 cm. below costal margin; liver not palpable	Patient previously treated with phenylhydrazine; symptomatic relief not as good as following P^{32}; delivered normal baby nine months after last injection of P^{32}

APPENDIX TABLE 1. CLINICAL RESULTS IN

PATIENT	BEFORE TREATMENT WITH P32		AFTER P32
	SYMPTOMS	PHYSICAL SIGNS DUE TO POLYCYTHEMIA	SYMPTOMS
13	Redness of eyes and lacrimation, blurring of vision, itching, soreness and bleeding of gums	Skin slightly redder than normal; spleen 6 cm. below costal margin; liver 3 cm. below costal margin	All original symptoms relieved except blurring of vision (glasses checked and found to be correct)
14	"Dazed feeling," headaches and shooting pains in head, aching in extremities, itching of skin, fatigability, palpitation; blind in left eye for 2 yr.	Skin and mucous membranes bright red; slight cyanosis; left optic atrophy; moderate pulmonary emphysema; B.P., 150/90; spleen 9 cm. below costal margin; liver 6 cm. below costal margin	No symptoms except very mild headaches and occasional dizzy spells; mild itching now confined to face only; blindness of left eye unchanged
15	Severe constant burning of tongue and bitter taste in mouth, moderate itching of skin, fatigability	Skin and mucous membranes bright red; "erythema multiforme" on trunk; conjunctivae red; spleen edge felt just at costal margin; liver not palpable; B.P., 150/80	Itching of skin and fatigability disappeared entirely; burning of tongue and bitter taste now bothers her only intermittently
16	Constant headaches, weakness and fatigue, deep pain in both legs, severe itching, pain and burning in eyes, clumsiness of left arm and leg	Skin and mucous membranes brilliant red; cyanosis; blotchy erythema on chest; conjunctivae red; spleen tip palpable just at costal margin; liver not palpable; B.P., 150/90	Patient now has only rare headaches and occasional slight aching in thighs; all other symptoms completely relieved
17	Severe pain in both legs, burning of eyes and lacrimation, epistaxis, nervousness, excessive perspiration	Skin and mucous membranes moderately red; slightly cyanotic; conjunctivae red; venules distended (fundi); spleen 11 cm. below costal margin; feet wet and cold	All symptoms completely relieved
18	Fatigability, headaches, dizzy spells, itching, aching and numbness of extremities, blurring of vision	Skin and mucous membranes slightly redder than normal; spleen palpable right at the costal margin	Patients still has occasional mild headaches, rare dizzy spells, and pains in legs
19	Nervousness for many years, dizziness and nausea (1 yr.), severe chorea and inability to talk coherently (1 mo.)	Face red; heart moderately enlarged; apical systolic murmur; spleen tip palpable on inspiration	Patient still somewhat nervous; all other symptoms completely relieved
20	Languor, drowsiness, difficulty concentrating, lack of interest in his work, blurring of eyes and of vision	Quite thin; mucous membranes bright red; marked tortuosity and distention of vessels in fundi; spleen palpable right at costal margin	All symptoms completely relieved for 6 mo.; drowsiness and lack of interest then returned (blood still normal)
21	Partial right hemiplegia (6 wk.), incoherent speech, dyspnea, blurring of vision, severe depression	Skin and mucous membranes slightly ruddy; right arm paralyzed; right facial paralysis; speech incoherent; pulmonary emphysema; spleen felt at costal margin	Dyspnea, blurring of vision, and depression have completely cleared up; speech much improved; right arm still paralyzed
22	Severe headaches, marked weakness and fatigability	Skin and mucous membranes bright red; cyanosis; B.P., 170/115; spleen not palpable	All symptoms completely relieved

THIRTY PATIENTS WITH POLYCYTHEMIA—CONT'D

THERAPY PHYSICAL SIGNS	REMARKS
No abnormal findings; liver and spleen not palpable	One month after last injection of P^{32} patient stopped menstruating; ever since then she has had hot flashes and nervousness; venesection (for 4 mo. prior to P^{32}) relieved most of symptoms
Color normal; optic atrophy and pulmonary emphysema as before; B.P., 130-150/80-90; spleen not palpable; liver 2 cm. below costal margin	Patient previously treated by phlebotomies with partial relief of symptoms; patient had been unable to work for 10 yr. prior to P^{32} therapy; now works 11-12 hours a day on his farm
No abnormal findings except B.P., 145-150/80; spleen not palpable	For years, patient had made it a practice to rub red pepper on tongue to relief the bitter taste; sometimes did this several times a day
No abnormal findings except B.P., 140-150/80-90	8 mo. before P^{32} therapy was started he had a sudden paralysis of left arm and leg; this gradually improved during period of several months, leaving only slight clumsiness
No abnormal findings; spleen not palpable	Patient previously treated with phenylhydrazine with very poor results; then controlled by repeated phlebotomies which gave temporary relief from all symptoms except severe pain in legs
No abnormal findings; spleen not palpable	1 phlebotomy (250 c.c.) performed 1 mo. after last injection of P^{32} (first course); ? pains in legs due to varicosities
No abnormal findings except an apical systolic murmur	Patient's mother had polycythemia; 6 wk. prior to P^{32} therapy patient had 4 x-ray treatments; 3 wk. prior to P^{32} therapy 5 phlebotomies (totaling 2300 c.c.) performed; chorea improved before P^{32} given
Gained 10 pounds; color normal; spleen not palpable	Previously treated with phenylhydrazine and venesections with little relief of symptoms; recent return of symptoms due to severe depression brought on by fiancee breaking engagement
Color normal; speech still difficult to understand but greatly improved; spleen no longer palpable; other physical signs unchanged	Patient treated with phenylhydrazine for several weeks and 2 phlebotomies performed before patient came to St. Louis for treatment; 3 additional phlebotomies performed just prior to P^{32} therapy
Color normal; B.P., 180-220/120-135	2 phlebotomies (450 c.c. each) performed, 1 on day P^{32} given and other 2 days later; EKG showed evidence of severe myocardial damage

APPENDIX TABLE 1. CLINICAL RESULTS IN

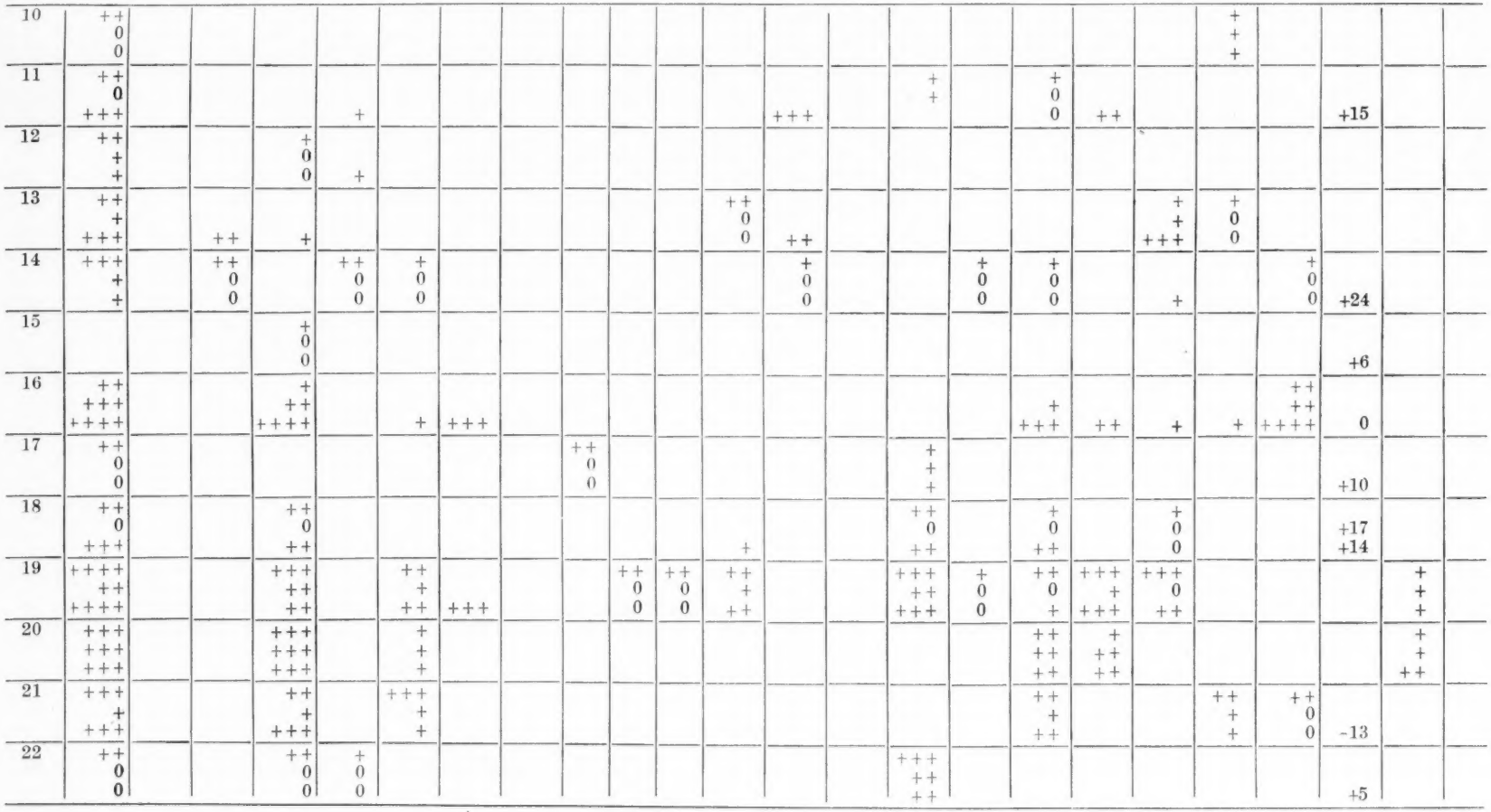
PATIENT	BEFORE TREATMENT WITH P^{32}		AFTER P^{32}
	SYMPTOMS	PHYSICAL SIGNS DUE TO POLYCYTHEMIA	SYMPTOMS
23	**Severe** headaches, dizziness, itching, dyspnea, palpitation, attacks of substernal pain associated with breathlessness, fatigability	**Skin** and mucous membranes bright red; cyanosis; conjunctivae red; B.P., 150/100; heart moderately enlarged; apical systolic murmur; expiratory wheezes throughout both lungs; spleen 5 cm. below costal margin; liver 10 cm. below costal margin	All previous symptoms cleared up entirely except itching which decreased greatly in intensity; however, patient now has occasional uncontrollable "crying spells" and loss of libido
24	Mild headaches, dizzy spells, fatigability, aching in thighs and legs, brief episodes of inability to move legs, frequent bruises, itching	Skin and mucous membranes bright red; conjunctivae red; venules greatly distended (fundi); B.P., 155/100; frequent premature systoles; spleen tip felt right at costal margin.	All symptoms greatly improved; only significant complaints are lack of pep and mild itching
25	Mild headaches, dizzy spells, aching pains in extremities, drowsiness, itching, blurring of vision, epigastric pain and vomiting	Skin and mucous membranes red; cyanosis; conjunctivae red; vessels of fundi sclerotic; pulmonary emphysema; spleen tip felt 2 cm. below costal margin	Dizziness, aching in extremities, drowsiness, and blurring of vision cleared up entirely; headaches now mild and infrequent; itching and ulcer symptoms unimproved
26	**Drowsiness, uncontrollable yawning**, mild headaches, dizzy spells, itching, pains in back and thighs, numbness of hands and feet, frequent bruises, polyuria, nervousness, hot flashes	Skin and mucous membranes bright red; cyanosis; distention of venules (fundi); spleen 13 cm. below costal margin; liver 6 cm. below costal margin	Patient still bothered by slight drowsiness, an occasional dizzy spell, bruises, cramps in feet, and hot flashes; all other symptoms have cleared up; in general, greatly improved
27	Headaches, weakness and fatigability, dizziness, itching, tinnitus, repeated "attacks" characterized by severe vertigo, blurring of vision and rigor	Asthenic; malnourished; distention of venules (fundi); spleen 2 cm. below costal margin	All symptoms greatly improved but still has occasional mild headache; feels much stronger; no "attacks"
28	Severe headaches, profound fatigability, dyspnea on exertion, swelling of ankles	Skin and mucous membranes bright red; pronounced cyanosis; conjunctivae red; venules of fundi distended; spleen 2 cm. below costal margin; liver 3 cm. below costal margin; B.P., 150/100	All previous symptoms now completely relieved; now has pain and edema of right leg
29	Headaches, pain in legs, itching, blurring of vision, repeated "heart attacks" characterized by pain in chest and epigastrium, palpitation, dyspnea	Skin and mucous membranes bright red; cyanosis; heart and lungs normal; B.P., 130/80; spleen not palpable; clubbing of fingers	All symptoms completely relieved except for an occasional mild headache and dizzy spell
30	Fatigability, dyspnea, headaches, dizzy spells, aching in extremities, itching, smarting of eyes and lacrimation, indigestion	Spleen 8 cm. below costal margin; no other abnormal findings	All symptoms relieved

THIRTY PATIENTS WITH POLYCYTHEMIA—CONT'D

THERAPY PHYSICAL SIGNS	REMARKS
Color normal; B.P., 120-135/85-100; auricular fibrillation; lungs clear but breath sounds distant; spleen not palpable; liver 8 cm. below costal margin	During 3 wk. preceding first injection of P^{32} 8 phlebotomies done (3,900 c.c. blood withdrawn); during next 2 mo. 2 more phlebotomies done (500 c.c. each); EKG showed only flat T waves in first three leads
No abnormal findings except auricular fibrillation; spleen barely palpable on deep inspiration	
Color normal; spleen not palpable; other findings unchanged	Gastrointestinal x-rays showed pyloric obstruction
Skin normal; spleen tip palpable just at costal margin	8 yr. prior to first therapy with P^{32} patient had epigastric pain relieved by food; four years later had hematemesis; x-rays at this time showed duodenal ulcer
No abnormal findings	Previously treated by phlebotomies with partial relief of symptoms
No abnormal findings except pitting edema of right leg	Previously treated by phlebotomies with great improvement but not as good as following P^{32}; 2 yr. ago patient had typical peptic ulcer symptoms which cleared up on diet and alkalies; 4 phlebotomies (500 c.c. each) done during first 2 wk. of P^{32} therapy; developed thrombosis of deep veins of right leg 4 days after P^{32} therapy
Skin and mucous membranes still redder than normal; cyanosis less marked; clubbing of fingers as before	Previously treated with phenylhydrazine and phlebotomies without significant relief of symptoms; patient apparently had a coronary occlusion 4 yr. previously, confirmed by EKG; patient has only been followed for 3 mo. since initial injection of P^{32} was given, and blood counts have not yet returned to normal
Spleen smaller (actual measurements not taken); no other abnormal findings	Patient was treated for 1 yr. by phlebotomies (500 c.c. withdrawn every 10-14 days); last phlebotomy 10 days before P^{32} injections; each phlebotomy relieved symptoms for about 1 wk.

APPENDIX TABLE 2. MYELOGENOUS LEUKEMIA—SYMPTOMATIC RESPONSE TO THERAPY

PATIENT	WEAKNESS AND FATIGABILITY	POUNDING IN HEAD	DIZZINESS	DYSPNEA ON EXERTION	PAIN IN EXTREMITIES	PALPITATION	BLEEDING—VAGINA, NOSE OR GUMS	SORENESS OF GUMS	SPONTANEOUS BRUISES	SEMICONSCIOUSNESS OR STUPOR	NIGHT SWEATS	CHILLS AND FEVERISHNESS	HEADACHES	TINNITUS	NERVOUSNESS	DIARRHEA	BLOATING, DYSPEPSIA	ANOREXIA	NAUSEA AND/OR VOMITING	ABDOMINAL PAIN	SWELLING OF LEGS, GENITALS	WEIGHT	COUGH	BLURRING OF VISION
1	++ 0 +++				0 0 0		0 0 +	0 0 +++		0 0 ++														
2	++ 0 +++	0 0 ++			+ 0 +++																			
3	+ 0 0			+ 0 0																				
4	+ 0 +++						+ 0 ++					+ 0 0	++ 0 0											
5	++ + +					+ 0 0									+++ ++ ++		++ + +	+ 0 +	+ 0 +					
6	+++ + +++		++ 0 0	++ + +			++ 0 +						++ 0 0	++ 0 0			+ 0 +	+ 0 ++						
7	++ 0 0	++ 0 0																+ 0 0	+ 0 0			+29		
8	+++ + +++				+ 0 0							++ 0 0						++ + +	++ 0 0	++ + ++		!		
9	+++ + +																+ 0 0	+ 0 0				!		



APPENDIX TABLE 2. MYELOGENOUS LEUKEMIA—SYMPTOMATIC RESPONSE TO THERAPY—CONT'D

PATIENT	WEAKNESS AND FATIGABILITY	POUNDING IN HEAD	DIZZINESS	DYSPNEA ON EXERTION	PAIN IN EXTREMITIES	PALPITATION	BLEEDING—VAGINA, NOSE OR GUMS	SORENESS OF GUMS	SPONTANEOUS BRUISES	SEMICONSCIOUSNESS OR STUPOR	NIGHT SWEATS	CHILLS AND FEVERISHNESS	HEADACHES	TINNITUS	NERVOUSNESS	DIARRHEA	BLOATING, DYSPEPSIA	ANOREXIA	NAUSEA AND/OR VOMITING	ABDOMINAL PAIN	SWELLING OF LEGS, GENITALS	WEIGHT	COUGH	BLURRING OF VISION
23	+ + + 0 + + -			+ + + + + + +		+ 0 0	+ 0 0														+ + + 0 +	+16	+ + + +	
24	+ + + + + + +			+ 0 + + +		+ 0 +						+ + + + +			+ + 0 + +		+ + + +	+ + + + + + +	+	+ + 0 + +		+5	+ + +	
25	+ + + + + -		+ + + + + +	+ + + +								+ + + +					+ + + + + +	+ +	+ + + +	+ + + +		No chg.		
Expired on third day of treatment																								
26	0 0 0																							
27	0 0 + +																0 0 +	0 0 + +				+10 +5		
28	+ + +			+													+	+ +			+	No chg.		
Expired on fifth day of treatment																								
29	+ + + + + +				+ 0 0		+ 0 0								+ + + + + +					+ 0 0		+25		
30	+ + + 0 0						+ 0 +						+				+ + 0 0	+ 0 0				+33		
31	+ + 0 0		+ + +	+ + 0 0		+ + 0 0															+ 0 0	+13		

32	++ 0 0			++ 0 0														++ 0 0	+ 0 0				+2		+ 0 0
33	+++ 0 ++				++						+	++						+++ + +++				+ 0 0	+8		
34	+++ 0 +						++ 0 0		++ + +									+++ 0 0		++ 0 0		++ 0 0			
35	+++ 0 0				++ 0 0																+ 0 0				+ 0 0
36	+ 0 0						+ + +		+ 0 0									++ 0 0			+ 0 0				
37	+++ 0 0	++ 0 0			+ 0 0		+ 0 0											++ 0 0			+ 0 0		+1 No chg.		
38	+++ ++ ++		++ + +					+ 0 0	+ + +														-3		
39	+++ 0 0															+++ + +			+ 0 0				+2		

The upper row of figures in each column represents the symptoms present at the time P^{32} therapy was started; in the second row is recorded the maximum improvement observed in each symptom during the first year of P^{32} therapy; in the third row are recorded the symptoms at the end of the first year of treatment or when last seen (if the patient has been followed for less than one year) or just prior to death (if the patient died in less than one year).

Zero indicates that the symptom was not present. If a symptom was present, the severity of the symptom is recorded as follows: Mild = +; moderate = ++; severe = +++; very severe = ++++. Blank spaces indicate symptoms were never present.

APPENDIX TABLE 3. MYELOGENOUS LEUKEMIA—CHANGE IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY

PATIENT	FEVER (1)	TACHYCARDIA (2)	PETECHIAE OR ECCHYMOSES (3)		BLEEDING FROM NOSE OR GUMS (4)	ENLARGED LYMPH NODES (5)	SYSTOLIC CARDIAC MURMUR (6)	SPLENOMEGALY (7)	HEPATOMEGALY (7)	EDEMA OF EXTREMITIES	SKIN ERUPTION (OTHER THAN PETECHIAE)	ASCITES	OTHER PHYSICAL SIGNS
			SKIN	MUCOUS MEMBRANES, FUNDI, OR CONJUNCTIVAE									
1	0	0	0	0	0	+	0	4-8	x-2	0	0		
	0	0	0	0	0	+	0	3-6	x-x	0	0		
	++	+	++	+	+	++	0	5-11	3-9	0	0		
2	0	0	0	0	0	0	0	x-x	x-x	0	++		
	0	0	0	0	0	0	0	x-x	x-x	0	+		
	+	+	+	+	+	++	0	3-7	x-x	0	+		
3	0	0	0	0	0	0	0	x-2	x-2	0	0		B.P., 155/100
	0	0	0	0	0	0	0	x-x	x-x	0	0		
	0	0	0	0	0	0	0	x-(-2)	x-x	0	0		B.P., 185/110
4	+	0	0	0	0	+++	0	4-9	x-x	0	0	0	
	0	0	0	0	0	++	0	4-9	x-x	0	0	0	
	0	0	0	0	+	+++	0	4-9	x-x	+++	0	+++	
5	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
6	+	++	0	0	+	++	+	16-21	2-6	+++	0	0	
	+	+	0	0	0	0	0	6-12	0-0	+	0	0	
	++	++	0	0	+	+	+	18-23	2-6	+	+	0	
7	0	0	++	0	0	0	+	9-14	2-7	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
8	+	0	0	0	0	0	+	4-10	x-x	++++	0	++	
	+	0	0	0	0	0	0	1-4	x-x	+	0	0	
	+	0	0	0	0	0	+	2-6	x-x	+	0	0	
9	0	0	0	0	0	0	0	6-10	1-4	0	0	0	B.P., 150/100
	0	0	0	0	0	0	0	x-1	x-x	0	0	0	
	0	0	0	0	0	0	0	x-1	x-x	0	0	0	
10	+	0	0	0	0	+	0	5-10	4-7	0	0	0	
	0	0	0	0	0	0	0	1-4	1-3	0	0	0	
	0	0	0	0	0	0	0	3-7	2-6	0	0	0	

11	0	0	0	0	0	+	0	9-16	1-3	0	0	0	
	0	0	0	0	0	0	0	2-5	x-x	0	0	0	
	++	+	+	0		0	0	13-23	3-9	0	0	0	
12	0	0	0	0	0	0	+	6-10	2-7	+	0	0	
	0	0	0	0	0	0	0	0-0	x-x	0	0	0	
	0	0	0	0	0	0	0	0-0	x-x	++	0	0	
13	++	++	0	0	0	+	+	3-8	1-4	0	0	0	
	0	0	0	0	0	0	+	x-0	1-4	0	0	0	
	+	++	0	0	0	0	+++	2-5	1-4	0	0	0	B.P., 170/90
14	++	++	+	0	0	+	+	22-31	15-20	0	0	0	
	0	0	+	0	0	+	0	9-12	4-9	0	0	0	
	0	+	0	0	0	+	0	10-16	6-10	0	0	0	
15	0	0	0	0	0	+	0	2-7	x-0	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	x-(-2)	x-x	0	0	0	
16	0	0	0	0	0	+++	+	4-8	5-9	++	0	0	
	+	+	0	0	0	++++	+	3-7	5-9	++++	0	0	
	+	+++	0	0	0	++++	+	?	?	++++	0	+++	
17	+	+	++	+	0	0	+++	2-6	x-x	0	0	0	
	0	+	0	0	0	0	++	x-x	x-x	0	0	0	
	0	+	+	0	0	0	++	x-(-2)	x-x	0	0	0	
18	0	+	0	+	0	+	0	10-18	8-13	0	0	0	
	0	+	0	0	0	0	0	x-x	x-x	0	0	0	
	+	++	+++	+	++	+	0	10-16	7-11	0	0	0	
19	++	+++	0	+	0	0	0	10-14	5-8	+	0	0	
	+	+++	0	0	0	0	0	4-9	2-5	0	0	0	
			+++	++	++	+++	++	8-13	3-5	0	0	0	Jaundice
20	++	+++	0	0	0	++	0	17-28	2-5	0	0	0	
	++	++	0	0	0	++	0	18-29	2-5	0	0	0	
	++	++	0	0	0	++	0	18-29	2-5	0	0	0	Consolidation, both bases
21	+	+	0	0	0	++	+	13-22	10-12	0	0	0	
	0	0	0	0	0	+	0	9-17	4-7	0	0	0	
	+	++	0	0	0	+	+++	11-21	8-10	0	0	0	
22	0	0	+	0	0	0	0	2-5	1-4	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	x-0	x-1	0	0	0	
23	0	0	0	0	0	+	+	10-14	4-8	++	0	++	
	0	0	0	0	0	0	0	2-6	x-x	0	0	0	
	+++	+++	0	0	0	+	+	8-15	x-x	+	0	+	Cardiac enlargement

APPENDIX TABLE 3. MYELOGENOUS LEUKEMIA—CHANGE IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY—CONT'D

PATIENT	FEVER (1)	TACHYCARDIA (2)	PETECHIAE OR ECCHYMOSES (3)		BLEEDING FROM NOSE OR GUMS (4)	ENLARGED LYMPH NODES (5)	SYSTOLIC CARDIAC MURMUR (6)	SPLENOMEGALY (7)	HEPATOMEGALY (7)	EDEMA OF EXTREMITIES	SKIN ERUPTION (OTHER THAN PETECHIAE)	ASCITES	OTHER PHYSICAL SIGNS
			SKIN	MUCOUS MEMBRANES, FUNDI, OR CONJUNCTIVAE									
24	0	+	0	0	0	+	+	5-9	5-8	0	0	0	
	0	0	0	0	0	0	0	2-4	3-5	0	0	0	
	+++	+++	0	0	0	+++	+	8-10	4-6	0	0	0	
25	+++	+++	0	0	0	0	+++	11-16	14-20	0	0	0	
	+++	+++	0	0	0	0	+++	11-16	14-20	0	0	0	
	Expired on third day of treatment												
26	+	++	0	0	0	+	0	x-x	x-x	0	0	0	
	0	+	0	0	0	0	0	x-x	x-x	0	0	0	
	0	+	0	0	0	0	0	x-x	x-x	0	0	0	
27	0	0	+	0	0	+++	0	6-11	x-x	0	0	0	
	0	0	0	0	0	+	0	5-10	x-x	0	0	0	
	0	0	0	0	0	+	+	16-24	?-6	0	0	0	
28	0	0	0	0	0	+	++	9-12	4-6	0	0	0	
	+	+	0	0	0	+	++	9-12	4-6	0	0	0	
	Expired on fifth day of treatment												
29	+	0	0	0	0	+	+	9-15	5-5	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
30	0	+	0	0	0	0	++	11-19	8-11	0	0	0	
	0	0	0	0	0	0	+	4-7	3-6	0	0	0	
	0	0	0	0	0	0	0	4-8	5-8	0	0	0	
31	0	+	0	0	0	0	+	1-x	0-x	0	0	0	
	0	0	0	0	0	0	0	x-x	x-x	0	0	0	
	0	0	0	0	0	0	0	(-2)-x	x-(0)	0	0	0	
32	0	++	0	0	0	+++	0	8-13	5-9	0	0	0	
	0	0	0	0	0	++	0	(-2)-x	x-x	0	0	0	
	0	0	0	0	0	++	0	(-2)-x	x-x	0	0	0	

33	0	+	0	0	0	+	0	15-23	5-7	0	0	0	
	0	0	0	0	0	+	0	11-18	3-5	0	0	0	
	+++	+++	0	0	0	+	0	18-23	3-5	0	0	0	
34	+	+++	0	0	+	++	++	12-18	8-10	0	0	0	
	0	+	0	0	0	0	0	x-x	x-x	0	0	0	
	0	+	0	0	0	0	0	x-x	x-x	0	0	0	
35	0	+	+	0	0	++	++	7-10	6-7	0	0	0	
	0	0	0	0	0	0	0	x-x	x-2	0	0	0	
	0	0	0	0	0	0	0	x-x	x-2	0	0	0	
36	0	+	0	0	0	+	0	10-16	x-x	0	0	0	
	0	0	0	0	0	+	0	(0)	x-x	0	0	0	
	0	0	0	0	0	+	0	(0)	x-x	0	0	0	
37	0	+	0	0	0	++	0	10-19	3-6	0	0	0	
	0	0	0	0	0	+	0	4-10	3-5	0	0	0	
	0	0	0	0	0	+	0	4-10	3-5	0	0	0	
38	0	0	+++	0	+	+++	++	7-9	3-5	0	0	0	
	0	0	+++	0	0	+++	+	(-2)-x	3-4	0	0	0	
	0	0	+++	0	0	+++	0	(-2)-x	3-5	0	0	0	
39	0	0	0	0	0	+	0	7-13	2-6	0	0	0	
	0	0	0	0	0	0	0	4-8	x-x	0	0	0	
	0	0	0	0	0	0	0	4-8	x-x	0	0	0	Thyroidectomy scar

The upper row of figures in each column represents the physical signs at the time P^{32} therapy was started; in the second row is recorded the maximum improvement in each physical sign during the first year of P^{32} therapy; in the third row are recorded the physical signs at the end of the first year of treatment or when last seen (if the patient has been followed less than one year) or at death (if the patient died in less than one year).

(1) Fever: Less than 37.5° C. = 0; 37.5° to 38.5° C. = +; 38.5° to 39.5° = ++; 39.5° to 40.5° C. = +++; above 40.5° C. = ++++ (average maximum daily temperature).

(2) Tachycardia: Less than 80 = 0; 80 to 100 = +; 100 to 120 = ++; 120 to 140 = +++; above 140 = ++++ (average maximum daily pulse).

(3) Graded 0 to ++++ or rough estimate of number and size of petechiae present.

(4) + = Present; 0 = absent.

(5) 0 = no palpably enlarged nodes; shotty nodes up to 0.5 cm. in diameter = +; nodes up to 1 cm. in diameter = ++; nodes 1 to 3 cm. in diameter = +++; nodes more than 3 cm. in diameter = ++++.

(6) Very loud murmur = +++; loud murmur = ++; faint murmur = +; no murmur = 0.

(7) Figures give actual measurement of spleen or liver edge below the costal margin in the anterior axillary line and in mid-clavicular line.

APPENDIX TABLE 4. LYMPHATIC LEUKEMIA—SYMPTOMATIC RESPONSE TO THERAPY

PATIENT	WEAKNESS AND FATIGABILITY	POUNDING IN HEAD	DIZZINESS	DYSPNEA ON EXERTION	PALPITATION	HEADACHES	SPONTANEOUS BRUISING	BLEEDING FROM VAGINA, NOSE OR GUMS	BLOATING, DYSPEPSIA	NAUSEA AND/OR VOMITING	ANOREXIA	ABDOMINAL PAIN	COUGH	PAIN IN EXTREMITIES	SORENESS OF GUMS	EXCESSIVE SWEATING	SWELLING OF LEGS OR GENITALS	WEIGHT
1	++ 0 ++++	++	++	++				++			++						+	
2	+++ ++ ++	+ + +	++ ++ ++	++ ++ ++	+ 0 +	+++ +++ +++								++ 0 ++		++ 0 0		
3	++ 0 0					+ 0 0		+ 0 0						+ 0 0				
4	+++ +++ ++++							++ ++ +++			++ ++ +++		+ 0 0	+ 0 0				
5	+++ 0 +++		+ 0 +											+++				
6	++ 0 0											++ 0 +		++				
7	+ 0 +++			+ 0 ++								+ 0 +						
8	++ + +			+ 0 0									+ 0 0			+ 0 0		
9	+++ ++ ++									++ ++ ++	+++ +++ +++	++ ++ ++		+ ++ ++				
10	+ 0 0																	
11	+++ ++ +++			++++ ++ ++++														

+9
+14
+12
+5
-2
+2
-3
12
13
14
15
16
17
18
19
20
21
22
23
24

APPENDIX TABLE 4. LYMPHATIC LEUKEMIA—SYMPTOMATIC RESPONSE TO THERAPY—CONT'D

PATIENT	WEAKNESS AND FATIGABILITY	POUNDING IN HEAD	DIZZINESS	DYSPNEA ON EXERTION	PALPITATION	HEADACHES	SPONTANEOUS BRUISING	BLEEDING FROM VAGINA, NOSE OR GUMS	BLOATING, DYSPEPSIA	NAUSEA AND/OR VOMITING	ANOREXIA	ABDOMINAL PAIN	COUGH	PAIN IN EXTREMITIES	SORENESS OF GUMS	EXCESSIVE SWEATING	SWELL. OF LEGS OR GENITALS	WEIGHT
25	+++ + +			+++ + +	++ 0 0													- 1
26	++ ++ ++++		+ + ++			++ ++ ++		+ + ++++	+ + +		+ + +++			0 + +				
27	+++ + +++		++ ++ ++	+ 0 +++		++ 0 ++		+ 0 +	+++ + +		+++ + +		+++ ++ ++++					- 7
28	++ 0 0																	
29	+++ + +						++ 0 0											
30	++ ++ ++											++ 0 0						+ 1

The upper row of figures in each column represents the symptoms present at the time P^{32} therapy was started; in the second row is recorded the maximum improvement observed in each symptom during the first year of P^{32} therapy; in the third row are recorded the symptoms at the end of the first year of treatment or when last seen (if the patient has been followed for less than one year), or just prior to death (if the patient died in less than one year).

Zero indicates that the symptom was not present. If a symptom was present, the severity of the symptom is recorded as follows: Mild = +; moderate = ++; severe = +++; very severe = ++++.

APPENDIX TABLE 5. LYMPHATIC LEUKEMIA—CHANGES IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY

PATIENT	FEVER (1)	TACHYCARDIA (2)	PETECHIAE OR ECCHYMOSES* (3)		ENLARGED LYMPH NODES (4)	SYSTOLIC CARDIAC MURMUR (5)	SPLENOMEGALY (6)	HEPATOMEGALY (6)	EDEMA OF EXTREMITIES	SKIN ERUPTION (OTHER THAN PETECHIAE)†	ASCITES	OTHER PHYSICAL SIGNS
			SKIN	MUCOUS MEMBRANES, FUNDI OR CONJUNCTIVAE								
1					++++		3-8					Enlargement of tonsils ++++
					++		0-0					Enlargement of tonsils ++
	+++	+++	+++	++	+++		0-0	0-2	++		+	Enlargement of tonsils +++
2		+			++	++	0-4	0-2				B.P., 220/100
		+			0	++	0-3	0-0				
		+			0	++	0-2	0-0				B.P., 230/125
3					+++		x-x	x-x				
					++		x-x	x-x				
					+++		x-x	x-x				
4			+++	++	+	++	0-1	1-2				B.P., 150/70
			+++	++	0	++	0-1	2-4				
			++++	++	0	++	0-1	2-4				
5					+++		x-0	x-x				
					+		x-x	x-x				
	++				+		x-x	x-x				
6					++		3-8	x-x				
					+		2-5	x-x				
					+		3-8	x-x				
7					+++		4-8	2-5				
					+		2-5	1-3				
					+		2-5	1-3			++	
8				+	++++		4-9	1-4				
				0	++		2-5	x-x				
				0	+++		5-11	2-6				
9					+		x-x	x-x				
					++		* *	* *				
					+++		3-8	2-5				
10					+		x-x	1-3				B.P.,178/116
					+		x-x	x-x				
					+++		x-x	x-x				

APPENDIX TABLE 5. LYMPHATIC LEUKEMIA—CHANGES IN PHYSICAL SIGNS FOLLOWING P^{32} THERAPY—CONT'D

PATIENT	FEVER (1)	TACHYCARDIA (2)	PETECHIAE OF ECCHYMOSES (3)		ENLARGED LYMPH NODES (4)	SYSTOLIC CARDIAC MURMUR (5)	SPLENOMEGALY (6)	HEPATOMEGALY (6)	EDEMA OF EXTREMITIES	SKIN ERUPTION (OTHER THAN PETECHIAE)	ASCITES	OTHER PHYSICAL SIGNS
			SKIN	MUCOUS MEMBRANES, FUNDI OR CONJUNCTIVAE								
11		++	+	+	++++	++	12-14	9-13	++	+++	++	
		++	+	0	++++	++	12-14	10-12	+	+++	++	
		+++	+	0	++++	++	12-14	10-12	+	+++	+++	Pleural effusion
12					+++		0-1	x-x			+++	Leukemia cutis
					+++		0-(-1)	x-x			+	
					++++		0-(-1)	x-x			+	
13	+				+++		1-x	1-5			+++	Draining ears; leukemia cutis
	+				++		(-1)-x	0-1			++	
	+				++		(-1)-x	0-1			++	
14	0				+++		2-0	5-10				
	+				++		0-0	1-4				
	+				++++		2-4	2-5				
15	+				++++	++	1-5	x-0				
	+				++	++	(-1)-x	x-x				
	+				++	++	(-1)-x	x-x				
16	++				+++	+	2-6	0-2				
	+				++	+	x-x	x-x				
	+	++			++	+	x-x	x-x				
17			+		++		(-2)-0	x-x				
			0		++		x-x	x-x				
			0		+++		x-x	x-x				
18		+			++	+	17-25	5-8				
		+			++	+	17-25	5-7				
	+++	+++			++	+	16-23[a]	5-7	+			
19					++++	+	5-10	x-x				
					++		5-10	x-x				
	+	++			++		5-10	x-x	+			
20			++		+++	+	7-15	x-x				
			0		+++	+	4-8	x-x				
		++	0		+++	+	4-8	x-x	+++			
21			++		+++		0-x	x-x				Enlargement of tonsils +++
			0		+		x-x	x-x				Enlargement of tonsils ++
			?		?		?	?				

22			++		+		15-17	6-9				
			++		++		13-18	5-8				
		+	++	+++	++		15-18	7-10				
23					++++	+	6-12	6-10	++			Enlargement of tonsils ++
					++	0	x-x	x-x	0			Enlargement of tonsils +
					++[b]	0	x-x	x-x	0			Enlargement of tonsils +
24		+			++		x-x	x-x				
		+			+		x-x	x-x				
		+			+		x-x	x-x				
25		++		+++	+++	+	8-14	4-9				
		+		0	++	0	4-7	x-x				
		+		0	++	0	4-7	x-x				
26	0	0	0	0	0		x-x	x-x				
	0	0	0	0	0		x-x	x-x				
	+	++	++++	+++	0		1-x	x-1	+			
27			0		+++		8-15	4-9				Râles +
			0		++		4-8	4-9				Râles 0
			+		+++		6-10	7-10				Râles +++
28		+			+		x-x	x-x				B.P., 175/100
		+			0		x-x	x-x				B.P., 175/100
		+			0		x-x	x-x				B.P., 175/100
29		+	++		+		3-x	1-4				
		+	0		+		0-x	1-4				
		+	0		++		0-x	2-6				
30			+		+++	+	8-15	1-4				
			0		+++	+	7-13	1-4				
			0		+++	+	7-13	1-4				

In the upper row of figures in each column are recorded the physical signs at the time P^{32} therapy was started; in the second row is recorded the maximum improvement in each physical sign during the first year of P^{32} therapy; in the third row are recorded the physical signs at the end of the first year of treatment or when last seen (if the patient has been followed less than one year) or at death (if the patient died in less than one year).

(1) Fever: Less than 37.5° C. = 0; 37.5° to 38.5° C. = +; 38.5° to 39.5° C. = ++; 39.5° to 40.5° C. = +++; above 40.5° C. = ++++ (average maximum daily temperature).

(2) Tachycardia: Less than 80 = 0; 80 to 100 = +; 100 to 120 = ++; 120 to 140 = +++; above 140 = ++++ (average maximum daily pulse).

(3) Graded 0 to ++++ on rough estimate of number and size of petechiae present.

(4) 0 = no palpably enlarged nodes; shotty nodes up to 0.5 cm. in diameter = +; nodes up to 1 cm. in diameter = ++; nodes 1 to 3 cm. in diameter = +++; nodes more than 3 cm. in diameter = ++++.

(5) Very loud murmur = +++; loud murmur = ++; faint murmur = +; no murmur = 0.

(6) Figures give actual measurement of spleen or liver edge below the costal margin in the anterior axillary line, and in mid-clavicular line.

[a] X-ray therapy administered to spleen.
[b] X-ray therapy administered to inguinal nodes.

APPENDIX

REMARKS ON THE ASSAY OF RADIOACTIVE PHOSPHORUS

MARTIN D. KAMEN, PH.D.

The activity of any radioactive substance is referred to an absolute standard, the "curie." Any substance emitting the same number of primary particles per second as does one gram of pure elementary radium is said to possess an activity of one curie. The most recent value for the number of disintegrations experienced by one gram of radium per second is 3.47 times 10^{10}. The assay of a radiophosphorus preparation then involves the determination of the number of disintegrations P^{32} undergoes per second referred to this unit. The P^{32} radiation, unlike that of radium which emits alpha and gamma rays, consists almost wholly of beta particles exhibiting the usual continuous beta spectrum and possessing a maximum energy of 1.69 millions of electron volts (M.E.V.). Detection of beta-rays involves an experimental setup radically different from that required in work with alpha and gamma rays. It is apparent, then, that a beta-ray standard is needed with which P^{32} samples can be compared and which, in turn, can be calibrated properly with respect to the curie standard.

It is not a simple mater to translate into curies the response of the various measuring instruments available, since the beta-rays produce an effect which is dependent on the geometry employed and the amount of absorbing matter through which they pass. In addition, the energy with which the beta-rays enter the sensitive volume of the detector determines the efficiency with which they are detected. In this brief note, only methods widely practiced at present for assaying radiophosphorus will be described. It is obvious that development of newer types of detectors expedited by war research should greatly modify the procedures employed in determinations of P^{32} dosage in future work.

Of the various detectors possible, two main types are employed: namely, electroscopes and Geiger counters. The voluminous literature on these instruments which has been made available in recent years renders unnecessary any discussion of them here or the principles underlying their use. The main type of electroscope used is the Lauritsen.* This instrument is a refinement of the familiar gold-leaf electroscope, the main improvement being the substitution of a gold-covered single fiber of quartz for the gold-leaf. The electrostatic capacity is about 0.2 centimeters and the charge sensitivity about 1 scale division for every 10^6 ion pairs. The motion of the quartz fiber is observed through a telescope ocular. In most cases, the instrument is supplied with a rather thick aluminum wall. To increase sensitivity to beta-rays, a portion of this wall is cut away and a thin aluminum foil ($\sim$ ½ mil thick) substituted.

A variety of Geiger counters is available, but since the samples are most conveniently mounted in dishes or flat planchettes, the open end "bell-jar" type

*See Lauritsen, C. C. and Lauritsen, T.: Rev. Scient. Instruments 8: 438, 1937.

of counter is most often employed. The Geiger counter is many orders of magnitude more sensitive than the Lauritsen electroscope but more complicated to maintain and operate. With either kind of detector two methods are available which one may label "absolute" and "differential." In the "absolute" method, the detector has a known response to the beta-rays and the activity can be read directly from the effect given by the detector. In the "differential" method, the response of the detector is unknown, but comparison is made using a standard with a known activity and with a radiation identical or closely similar to the P^{32} radiation, both standard and unknown being measured under identical conditions.

The most popular standard for use with the differential method is a radioactive daughter of uranium, namely, UX_2. In the uranium series, the parent uranium disintegrates by alpha emission with a half-life of 4.6×10^9 years to UX_1. The UX_1 in turn emits a low energy beta-ray (completely absorbed in $\sim$ 25 mg./cm. $\sim$ Al) with a half-life of 24.5 days decaying to UX_2. (A negligible fraction of UX_1 disintegrates to a nuclear isomer of UX_2 which is known as Uranium Z.) The beta radiation from UX_2 (half-life of 1.14 minutes) has a maximum energy of about 2.3 millions of electron volts and resembles quite closely that from P^{32}. Any sample of reagent uranium or uranium salt is, by virtue of these disintegration relations, in equilibrium with the radioactive daughters of uranium. Thus, a given weight of uranium can be computed to be the equivalent of a certain amount in curies of UX_2. By spreading a weighed amount of uranium or uranium salt in a dish similar to that used in determining the phosphorus and covering the radioactive substance with sufficient aluminum foil to cut out all but the UX_2 radiation, it is possible to devise a standard with a known activity in microcuries for direct comparison with the P^{32} sample. If a Lauritsen electroscope is used, the amount of uranium needed is large enough so that some UX_2 radiation is lost by self-absorption in the sample and a correction must be applied. Thus, to obtain a microcurie (10^{-6} curies), the quantity of uranium oxide (U_3O_8) required is 3.5 grams. When spread on a dish with an area of 10 cm.,^{2} this gives an absorption thickness of 350 mg./ cm..^{2} A sample one-tenth as strong as this is a convenient one for use with the electroscope. In this case one must correct for an absorption of 35 mg./cm.^{2} The absorption of the beta-rays is logarithmic, half-value corresponding to approximately 110-120 mg./cm.^{2} in aluminum. The absorption correction must be determined separately in each laboratory since the curve is a function of the particular electroscope and geometry employed. Thus, the scattering of the electrons in the material of the dish and adjacent objects affects markedly the absorption curve obtained. In working with Geiger counters, much thinner samples can be used because of the greater sensitivity of the counters. In any case, a correction for the aluminum foil used to exclude UX_1 radiation is essential.

Such a standard has been employed mainly by the laboratories at the University of California and at Washington Uinversity. A recent cross-check of the standards used in these two laboratories shows that agreement within 25 per cent in the absolute value of the microcurie obtains, the St. Louis value

being the lower. Recently, I have found that about two-thirds of this discrepancy can be explained by inhomogeneities in spreading of the P sample with the particular geometry used at St. Louis. Thus, the St. Louis value now agrees with the California microcurie within 10 per cent.

Radiophosphorus supplied by the Radiation Laboratory at Massachusetts Institute of Technology is assayed by the "absolute" method. To obtain a Geiger counter which has a known response to beta-rays, a radioactive substance with known disintegration scheme emitting both beta and gamma rays is measured, using the counter to determine coincidences between beta and gamma rays. By using several such substances and a variety of absorbers, it is possible to determine the variation of counter efficiency with beta-ray energy. This counter, once calibrated in this fashion, is used to determine the counting rate of a Radium E source in equilibrium with its parent 22 year Radium D. This serves as a reference source to check variations in counter efficiency from day to day. The microcurie standard determined in this manner at M.I.T. is smaller than those at either Berkeley or St. Louis by a factor of 2.4. Thus, a sample of radiophosphorus measured in St. Louis as 100 microcuries is measured at M.I.T. as 240 microcuries at present. This discrepancy should be borne in mind when response to therapeutic dosages in different laboratories is compared. The reason for this discrepancy is obscure. Nothing in the present procedures used indicates an error in either method of more than 10 per cent. In preparing samples at Berkeley and St. Louis, an aliquot of the P^{32} solution is pipetted into a bottle cap or porcelain dish, evaporated, and mounted under the detector window. The standard prepared in precisely the same kind of holder and mounted in the same place is measured in identical fashion. The ratio of the two readings gives directly the strength of the P^{32} sample. In the M.I.T. method, the aliquots are pipetted onto paper planchettes, dried, and placed in contact with the counter window. The RaD standards are prepared and assayed in precisely the same fashion. The ratio of the readings corrected for the efficiency of the counter to P^{32} radiation energy gives the strength of the P^{32} sample.*

Investigations are now proceeding at all three institutions to eliminate the discrepancy. Since the prospect of a rapid expansion in radiophosphorus therapy is not remote, it would appear advisable to make available a standardized procedure which is simple and reliable and in which there is agreement among all workers in the field. Prescriptions for precision beta-ray standards resulting from these investigations should make precision standards available.

*The writer is indebted to Prof. J. I. Irvine, Jr., for a description of the method employed at M. I. T.

THE TREATMENT OF ULCERATIVE COLITIS WITH NISULFADINE AND NISULFAZOLE

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ULCERATIVE colitis is a disease of uncertain etiology. In 1931 Hurst stated that in his opinion "the disease is a form of bacillary dysentery." However, some ten years later, after a study of both chronic bacillary dysentery and chronic ulcerative colitis in North China, Snapper stated flatly that "as far as x-ray evidence goes, chronic ulcerative colitis must certainly be distinguished from chronic bacillary dysentery."

In 1924 Bargen reported the presence of a gram-positive diplostreptococcus in 80 per cent of the cases of chronic ulcerative colitis at the Mayo Clinic. He thought this finding of etiologic importance. However, to quote Willard, "Most observers have been unable to confirm Bargen's findings in any large percentage of cases." Paulson, Dukes, Mackie, and Kessel have criticized Bargen's work. Kessel, in his studies on the bacteria found in the colon of normal individuals and in patients with ulcerative colitis, states, "There would, therefore, appear to be no room to regard the streptococci described as Bargen's diplostreptococcus as being essentially different from other types of alpha streptococci commonly recovered from the human alimentary tract and his strains may belong to the species *Streptococcus fecalis, Streptococcus mitis,* or *Streptococcus salivarius.*"

Another claimant for the role of etiologic agent is the *Bacterium necrophorum,* studied particularly by Dack and his co-workers. Their work is of great interest but awaits further confirmation.

The possible relationship of the virus of lymphogranuloma venereum, or of a similar virus, to ulcerative colitis has been suggested by several observers. Rodaniche, Kirsner, and Palmer in 1940 studied this problem in thirty-four cases of chronic ulcerative colitis. They concluded that "the fact that thirty-two out of thirty-four patients (94 per cent) with chronic ulcerative colitis and proctitis in this series had negative Frei tests and showed no evidence of neutralizing antibodies in their serums against the virus of lymphogranuloma venereum indicates that this virus was in no way involved in the great majority of cases."

The role of psychogenic factors in the etiology of chronic ulcerative colitis has been stressed by many writers. The emotional instability of many of these patients is a fact beyond dispute. However, to assign to it a role beyond that of a predisposing cause would require more proof than has yet been brought forward. Allergy and dietary deficiencies have been suggested by some as

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etiologic factors. Here again, a definite relationship is difficult to prove, although dietary deficiencies are frequently seen in these patients just as they are in other patients suffering from entirely different diseases.

If we find the literature on the subject of the etiology of chronic ulcerative colitis confusing and unconvincing, a review of the methods proposed for the treatment of this disease offers little. The therapeutic procedures proposed (vitamins, artificial fever, oxygen, vaccines, serums, and rectal instillations) are a clear indication that none of them are adequate. The only possible exception is therapy with sulfanilamide and related drugs, where encouraging results have been reported by several observers, notably by Bannick, Brown, and Foster.

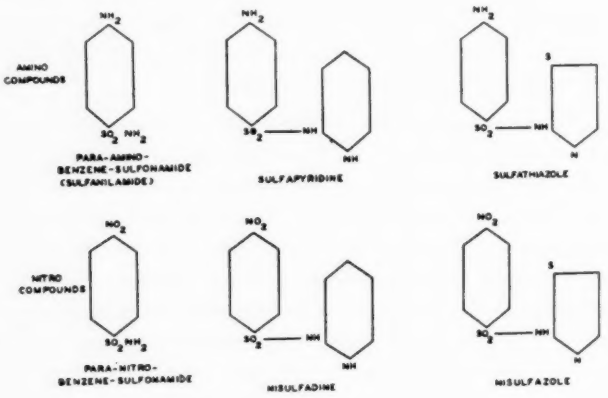

Fig. 1.

During the past four years we have treated twenty-one patients suffering from chronic ulcerative colitis with two new compounds which we have employed in several other studies. These compounds are 2-(p-nitrobenzene sulfonamido)-pyridine, which we shall call nisulfadine, and 2-(p-nitrobenzene sulfonamido)-thiazol, which we shall call nisulfazole. The relationship between sulfapyridine and nisulfadine and between sulfathiazole and nisulfazole is shown in Fig. 1. The physical properties and some of the reactions of these substances have been described in previous papers. A few of these properties may bear a certain relationship to their apparent therapeutic action.*

Nisulfadine is reduced in the intestinal tract to sulfapyridine; nisulfazole, to sulfathiazole. Most of this conversion takes place in the colon and may be the result of bacterial action. In test tube experiments, *Bacillus coli, Bacillus typhosus* and *Bacillus dysenteria* (Shiga, Flexner, and Hiss strains) produce a reduction of the nitro compounds when the concentration of these compounds does not exceed 4 mg. per cent. Approximately 50 per cent of the nisulfadine is converted into sulfapyridine and 50 per cent of the nisulfazole is changed to sulfathiazole. When the percentage of the nisulfazole in the broth was increased, it was found that the growth of the Hiss, Shiga, and Flexner strains of *B. dysenteria* was markedly inhibited, while *B. coli* and *B. typhosus* were

*Supplied through the courtesy of George A. Breon & Company, Inc., Kansas City, Mo.

little affected. Both nitro compounds, especially nisulfazole, produced a marked inhibition in the growth of *Streptococcus viridans, Str. fecalis,* and *Streptococcus hemolyticus.*

The results of the oral ingestion in dogs are shown in Tables I to IV. When nisulfadine is administered by mouth, approximately 25 per cent appears in the blood as sulfapyridine after four hours (Table I); with nisulfazole, only a faint trace of sulfathiazole appears in the blood after seven hours (Table II). This suggests that nisulfazole is less rapidly reduced and also less readily excreted by the urine. Observations on patients show similar results (Table III).

Our observations on the treatment of chronic ulcerative colitis with nisulfadine and nisulfazole were made on twenty-one patients, the small number of patients treated being due partly to the difficulty of obtaining sufficient supplies of these drugs.

Clinically, all of the patients had shown chronicity and a marked tendency to severe recurring attacks. In each case the clinical diagnosis was confirmed

TABLE I. BLOOD VALUES FOR SULFAPYRIDINE AND NISULFADINE IN THE DOG AFTER THE ORAL ADMINISTRATION OF NISULFADINE 4.35 GM.

BLOOD (HR.)	SULFAPYRIDINE (MG. %)	NISULFADINE (MG. %)
2	Trace	1.13
4	1.02	0.48
8	1.92	0.55
12	3.24	0.30
24	2.79	0.41

TABLE II. BLOOD VALUES FOR SULFATHIAZOLE AND NISULFAZOLE IN THE DOG AFTER THE ORAL ADMINISTRATION OF NISULFAZOLE 5 GM.

BLOOD (HR.)	SULFATHIAZOLE (MG. %)	NISULFAZOLE (MG. %)
2	None	0.59
4	Faint trace	0.75
7	Faint trace	4.30
24	2.0	2.30

TABLE III. BLOOD VALUES OF SULFAPYRIDINE, NISULFAZOLE, SULFATHIAZOLE, AND NISULFAZOLE IN PATIENTS

DATE	SULFAPYRIDINE* (MG. %)	NISULFADINE (MG. %)	DATE	SULFATHIAZOLE* (MG. %)	NISULFAZOLE (MG. %)
9/ 4/41	5.75	2.90	2/27/42	1.1	6.1
9/ 6/41	4.30	1.50	3/ 3/42	0.7	6.1
9/ 8/41	2.30	2.00	3/ 6/42	0.8	6.7
9/10/41	4.05	1.80	3/11/42	0.8	6.2
9/15/41	4.70	3.60	3/13/42	1.0	5.8
9/17/41	6.35	2.45	3/18/42	1.6	8.4
9/20/41	7.90	3.20	3/21/42	1.5	9.2
Patient received during this period an average dose of 3 Gm. nisulfadine daily			Patient received during this period 4 Gm. nisulfazole daily		

*Values calculated as sulfapyridine and sulfathiazole. Actually known only as compounds containing amino groups giving the characteristic color reaction with sodium nitrite.

by roentgenologic evidence and by the demonstration of ulcers on proctoscopic examination. We were impressed by two complications. First, severe anemia of the secondary type was observed in every patient and could not be explained by the loss of blood from the bowel. Severe anemia, as a concomitant finding in ulcerative colitis, has been stressed by several observers, notably by Schemensky. Second, four of our patients showed ulcerations on the skin, particularly on the arms and legs. One patient had such deep ulcers on the legs that contractures of the leg muscles appeared, necessitating orthopedic measures. This complication has been studied by many observers. Felsen reported three cases from which he isolated, respectively, *B. coli, Staphylococcus aureus* and a nonhemolytic streptococcus. All of our cases began with a small subcutaneous abscess, which gradually increased in size, then burst, discharging thick, creamy pus and leaving an ulcer. The pus, on direct smear, showed no organisms and the cultures made both aerobically and anaerobically, and in a variety of media, showed no growth.

Four of the patients are dead. One patient died of coronary occlusion two years after discharge from the hospital, having had no symptoms of colitis during the interval. Another patient died one month after cessation of all intestinal symptoms, the cause of her death being unknown. A third patient died from lobar pneumonia two years after dismissal from the hospital, apparently well. A fourth patient who also suffered from bronchiectasis died six months after dismissal from the hospital, the cause of death being probably pneumonia complicated by recurrence of ulcerative colitis. One patient was operated upon and an ileostomy performed. Under treatment with nisulfazole the number of stools before operation was reduced from a daily average of from eighteen to two.

All of the patients treated were greatly improved, including those who subsequently died. Seventeen of these patients are now free from symptoms and show normal roentgenograms; seven are markedly improved, four of these being still under observation and receiving treatment from time to time. The drug first used was nisulfadine, but later nisulfazole was employed exclusively since it produced less nausea. The initial dose was from 4 to 6 Gm. in twenty-four hours; with clinical improvement, this dose was reduced to 2 Gm. Reduction in the number of bowel movements was prompt and striking. In five patients, after two weeks of therapy, enemas were occasionally necessary to produce bowel movements. Prolonged medication over a period of months caused no untoward symptoms, although in several instances a reduction in the leucocyte count led us to discontinue medication for a brief interval.

The drug was administered in three forms. The patients received first the compound in tablets. In some instances the tablets soon produced nausea and in their place a pectin suspension of nisulfadine was employed. This was usually tolerated by the patient. In some patients, nisulfazole in a pectin suspension was administered by rectum. This method of administration was usually promptly effective and produced no nausea. More recently, enteric-coated tablets of nisulfazole have been employed. These are effective and apparently produce no nausea. However, while the patient has diarrhea, they

may pass through the intestinal tract unchanged because of the active peristalsis and are usually ineffective during the first days of the treatment. The method of administration may vary with the individual and a decision as to the best method awaits further experience.

In addition to drug therapy, the patients were placed on a diet with a minimum of residue and received vitamin therapy, especially B complex, and those with marked anemia were given blood transfusions.

In Table IV are given data on the patients studied in the Medical Clinic of the University of Kansas.

A few additional remarks should be made regarding the data given in Table IV. The term recovery is employed when the patient is symptom free and the x-ray picture shows a normal colon. The term improvement is employed to designate patients who have no diarrhea and have normal stools but show stenosis on thickening of the colon in the x-ray picture. Patients 14, 18, 19, and 20 are symptom free at present but still show a narrowed and constricted colon. These patients had suffered from chronic ulcerative colitis thirteen years, ten years, eighteen years, and twelve years, respectively. These changes in the colon are probably permanent.

The term slight relapse is employed in four instances where the patient developed a slight diarrhea and following instructions returned promptly to the hospital for treatment, to which they responded promptly.

The following are two typical case histories:

D. B., aged 37 years, was admitted to the University of Kansas Hospitals Aug. 4, 1942, complaining of diarrhea. The family history was negative. Past history: the patient had enjoyed good health up to the onset of the present illness, which began approximately six years before admission to the hospital, and since that time had had continued diarrhea which had resisted all types of therapy. The diarrhea had increased in severity through the years. During the past two months the diarrhea had been particularly severe, the patient having never less than eight and often as many as twenty stools in twenty-four hours.

Physical examination showed evidence of marked loss of weight. Proctoscopic examination showed mucous bleeding and numerous ulcerations in the rectum. The patient weighed 111 pounds. Admission blood count was 3,600,000 red cells, 6,700 white cells, and 68 per cent hemoglobin (10.5 Gm.). X-ray of the colon showed a colon devoid of haustrations, varying greatly in diameter, being narrow in the region of the splenic flexure and wide in the transverse and descending colon. There was a granular type of architecture throughout the colon from the hepatic flexure downward and feathering of the margin of the colon, and the conclusion of the roentgenologist was ulcerative colitis involving the transverse and descending colon and sigmoid.

The patient was started on nisulfazole, 1 Gm., six times daily, which was later reduced to four times daily. In addition to this treatment, she was given six blood transfusions during her stay in the hospital, which lasted to Oct. 11, 1942. At the time of her dismissal, she was very markedly improved, the stools within ten days having been reduced to not more than two in twenty-four hours.

The patient was followed further and has continued to improve since her dismissal from the hospital. On Dec. 13, 1942, she was readmitted for an acute abdominal condition, diagnosed as acute hemorrhage into round ligament and abdominal wall.

On May 11, 1945, she was readmitted to the hospital for observation. The blood count at this time was 4,120,000 red cells, 4,200 white cells, and 74 per cent hemoglobin. X-ray of the colon showed no narrowing of the colon and no evidence of ulceration. There

TABLE IV

PATIENT	AGE (YR.)	SEX	DURATION	ADMITTED	NUMBER OF STOOLS DAILY ON ADMISSION	NUMBER OF STOOLS DAILY ON DISMISSAL	LENGTH OF TREATMENT	RESULT	RELAPSE	REMARKS
1	71	M	18 mo.	6/23/41	10	2	1 mo.	Recovery	None	Died, "heart attack," 10/19/43
2	16	M	4 mo.	6/30/41	18	2	3 wk.	Recovery	One slight	Died, pneumonia, 1/18/44
3	23	M	14 mo.	12/14/41	14	2	5 mo.	Recovery	Two slight	Well, 2/15/44
4	19	F	9 mo.	1/19/42	10	2	4 wk.	Recovery	One slight	Well, 11/23/45; gained twenty pounds
5	22	F	5 mo.	2/13/42	27	3	2 mo.	Recovery	None	Died later, cause unknown; no diarrhea
6	19	M	30 mo.	7/23/42	8	3	3 mo.	Improvement	Two	Died, 12/21/44
7	37	F	12 mo.	8/ 4/42	20	2	10 wk.	Recovery	None	Well, 5/11/45; gained fifty-three pounds
8	18	F	6 yr.	1/16/43	7	1	1 mo.	Recovery	None	Well, 1/14/44
9	54	M	3 yr.	1/19/43	18	2	4 mo.	Improvement	None	Operation
10	40	M	5 mo.	8/ 2/43	8	2	1 mo.	Recovery	None	"Perfectly well," 8/24/45
11	41	F	1 mo.	10/24/43	10	1	10 wk.	Recovery	None	Well, "feeling fine," 5/18/45
12	37	F	4 mo.	3/14/44	6	1	3 mo.	Recovery	None	Well, 11/5/45; gained thirty pounds
13	24	M	18 mo.	7/20/44	10	2	2 mo.	Improvement	None	Improved, 9/24/45
14	13	M	5 yr.	8/22/44	12	2	1 mo.	Improvement	One	Improved, 10/21/45
15	30	F	3 yr.	10/22/44	15	2	2 mo.	Recovery	None	Well, 10/2/45
16	18	F	7 mo.	10/31/44	18	1	2 mo.	Recovery	One slight	Well, 1/18/46
17	27	F	4 yr.	11/25/44	20	2	4 mo.	Recovery	None	Well, 8/1/45; gained thirty pounds
18	14	M	10 yr.	1/24/45	8	2	1 mo.	Improvement	Two	Continued improvement, 10/1/45
19	55	M	18 yr.	3/ 1/45	8	2	7 wk.	Improvement	None	Well, 10/15/45; gained ten pounds
20	24	F	12 yr.	3/10/45	7	2	5 wk.	Improvement	None	Well, 10/1/45; gained fifteen pounds
21	44	M	11 yr.	4/14/45	4	1	3 wk.	Recovery	None	Well, 9/18/45

was still some lack of haustrations although this was not marked. The patient has continued to be free of diarrhea and weighs 164 pounds, a gain of fifty-three pounds since the time of her first admission to the hospital.

T. M., a Negro woman, was admitted to the University of Kansas Hospitals March 14, 1944, complaining of bloody diarrhea. The family history was negative. Past history: the patient had enjoyed general good health and had had no severe illnesses; described herself as being of a somewhat nervous disposition. The present illness began in December, 1943, when the patient began having bloody diarrhea with from eight to ten movements a day. This continued until admission to the hospital. She had become very anemic and lost fifteen or twenty pounds (weight now, 110 pounds).

On admission to the hospital the patient showed evidence of loss of weight; the mucous membranes were rather pale. The heart, lungs, and abdomen were negative on admission. The blood pressure was 85/65 and the pulse, 120. Examination of the rectum ten days after admission to the hospital showed numerous small ulcers.

On admission, the blood count showed 2,850,000 red cells, 13,000 white cells, and 55 per cent hemoglobin. Urinalysis was negative. X-ray examination showed complete lack of haustrations in the colon; along the medial contour of the descending colon definite feathering was seen, indicative of superficial ulceration. The conclusion of the roentgenologist was "active ulcerative colitis."

Treatment was started immediately and she received nisulfazole, 4 Gm. daily, with vitamin C, 1 Gm. In addition to this, she was given blood transfusions, five being given during her entire stay in the hospital.

Improvement was noticeable within forty-eight hours. The patient's bowel movements were reduced to three a day, and from March 20 until the time of her dismissal, the bowel movements never exceeded three in twenty-four hours.

At the time of her dismissal from the hospital May 24, 1944, the blood count was 4,190,000 red cells, 7,650 white cells, and 74 per cent hemoglobin. The report on the barium enema on that day was: "The colon fills normally. No pathology is seen. No evidence of ulceration is seen; neither is there scarring."

This patient has been followed since and continues in excellent condition. After leaving the hospital she continued taking 2 Gm. of nisulfazole daily, which was discontinued July 14, 1944. The blood count June 2, 1945, was 4,240,000 red cells, 6,000 white cells, and 81 per cent hemoglobin.

The patient's condition at the present time is excellent. A letter of Nov. 3, 1945, states: "I have one bowel movement a day; once in a while I won't have any."

In a disease such as chronic ulcerative colitis, a disease noteworthy for its chronicity and its tendency to recurrence, it would be hazardous to assert that most of these patients are permanently cured. We do feel, however, that therapy with nisulfazole has given us much better results than any other treatment with which we are familiar.

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OBSERVATIONS ON THE TREATMENT OF TROPICAL SPRUE WITH FOLIC ACID

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THE discovery of the antianemic effect of synthetic folic acid in certain types of macrocytic anemia in relapse opens up a new era in the study of the pathogenesis and therapy of these conditions.[1-3] Included in the group of patients so dramatically relieved by folic acid were those with Addisonian pernicious anemia and those with nutritional macrocytic anemia. Because the pathogenesis of none of the macrocytic anemias is thoroughly understood, there has been much confusion concerning their diagnosis. Our studies[4, 5] have shown that Addisonian pernicious anemia and nutritional macrocytic anemia are cytologically indistinguishable and that the best single differentiating feature is the presence of free hydrochloric acid in the gastric juice in persons with nutritional macrocytic anemia and the absence of free hydrochloric acid in the gastric juice of persons with Addisonian pernicious anemia even after histamine stimulation. We have found that the macrocytic anemia of pellagra and of pregnancy, from a laboratory point of view, are in no way distinguishable from nutritional macrocytic anemia, and all respond to folic acid. It seems wise, therefore, to abandon the terms "macrocytic anemia of pellagra" and "macrocytic anemia of pregnancy" and classify them as nutritional macrocytic anemias. We have also observed, as have many others, that cytologically the anemia of sprue and nutritional macrocytic anemia are identical. The relationship between the two diseases is very difficult to grasp, and differentiation may not be justifiable. The final answer to the question may have to wait until their etiology is more completely understood. Acid steatorrhea is a characteristic and specific feature of sprue, and we make a diagnosis of sprue rather than nutritional macrocytic anemia in the presence of steatorrhea.

Although macrocytic anemias occur throughout the world, certain types are found much more frequently in one area than in another. In the temperate zones, only sporadic cases of so-called nontropical sprue are seen, whereas in some of the tropical areas sprue is endemic. There is a difference of opinion among some physicians as to the pathogenesis of tropical and nontropical sprue, but we consider both as similar conditions. The number of cases of sprue seen in

From the University of Cincinnati Studies in Nutrition at the Calixto Garcia Hospital, Havana, Cuba, in cooperation with the Institute of Nutrition of Cuba and the University of Havana.

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the United States, however, is small; and, in order to study thoroughly the effectiveness of folic acid in treating the macrocytic anemia of tropical sprue, it seemed necessary to go to an area in the tropics. Through the cooperation and assistance of the University of Havana and the Institute of Nutrition of Cuba, a special ward was obtained at the University Hospital in Havana. Twenty-five patients were examined clinically and hematologically, and of this number nine were selected for the study.

SELECTION OF PATIENTS

In selecting the patients for the therapeutic assay of folic acid, the following criteria were used: (1) The patient had to have a macrocytic anemia; (2) the bone marrow had to show the typical erythroblastic arrest seen in pernicious anemia; (3) the erythrocyte counts had to be below 2.5 million; (4) the patient had to be untreated, or he must not have been treated recently enough to interfere in any way with our evaluation of the effect of folic acid; (5) he had to have persistently low reticulocyte counts during the period of observation; (6) he had to have glossitis and diarrhea characterized by fatty stools.

With these criteria in mind, we selected for study nine white patients (six men and three women) ranging in age from 29 to 75 years.

METHOD OF STUDY

All the patients were hospitalized for preliminary examination, base-line determinations, and treatment. A detailed medical and dietary history was taken and a complete physical examination was made in each case. From the time the patients were accepted for study, their diets were rigidly controlled. Meat, meat products, fish, and poultry were excluded, and only one quart of milk and one egg were allowed daily. Bread, cereals, fruits or vegetables, sugar, fats, and coffee were allowed in any amount desired. In a previous study of anemia, seventy-five patients were restricted to this type of diet, and none of them had a so-called spontaneous remission. We felt reasonably certain, therefore, that any hemopoietic response would be attributable to the folic acid rather than to any food the patients received.

Daily hematologic examinations included white and erythrocyte counts, hemoglobin determinations, and reticulocyte counts. Certified Trenner pipettes were used for both the white and erythrocyte counts. The hemoglobin content of the blood was determined in grams by means of a Leitz colorimeter, and the reticulocytes were counted in wet preparations by the use of a modified brilliant cresyl blue solution of Dameshek. From time to time cell volumes were determined on oxalated venous blood by means of Wintrobe hematocrit tubes. In each case bone marrow was obtained prior to treatment by sternal aspiration. Differential counts were made on preparations stained with both supravital and Wright-Giemsa stains. Gastric contents were collected before and after histamine injection in all patients.

In each case a twenty-four hour stool specimen was collected daily in a single large glass graduated container. The stools were examined every day for appearance and volume. Each patient was questioned every morning con-

cerning the number of bowel movements. Examinations of the feces included tests for acidity, fatty acid determinations by the method of Labbé and Larué, bacteriologic examinations, and examinations for parasites.

Serum protein determinations were made in each patient. In most cases blood calcium, phosphorus, potassium, amylase, and lipase determinations were made.

Dietary histories revealed that in each case the diet had been deficient in animal protein over a long period of time. The foodstuffs containing animal protein most frequently ingested were milk and occasionally eggs, but these were always consumed in inadequate quantities. Vegetables were rarely eaten and the only fruits eaten were mangos, avocados, and citrus fruit, and these only in small amounts. The diet consisted almost entirely of rice, corn meal, and *viandes* (root vegetables grown in Cuba).

Loss of body weight, which had occurred in all subjects, varied from fifteen to forty pounds. All patients complained of extreme weakness and fatigue on even slight exertion. During the preliminary period of observation, they lay quietly in bed. They had no apparent desire to move and manifested no interest in their surroundings. All the patients except one (Case 8) complained of loss of appetite and a distaste for food. Without exception, they complained of a feeling of fullness, flatulence, and abdominal distention after meals and of a burning sensation of the mouth and tongue which increased in severity while they were eating. This sensation sometimes extended to the throat and epigastrium after swallowing. In some patients rectal burning was described.

All the subjects gave a history of having had diarrhea for periods of time which ranged from four months to four years. The stools were light colored, large (from 200 to 1,200 c.c. a day), frothy, foul smelling, semiliquid or liquid, and varied in number from three to twenty a day. Bowel movements were accompanied by expulsion of large amounts of gas and by borborygmus. In many instances the bowel movements were preceded by colic.

Physical examinations revealed that in each case the skin was pale and dry with desquamating areas which varied in location in different patients but appeared most commonly on the arms, hands, and face. In some patients pigmentation was generalized. In four patients there were hemorrhagic diffusions of violet discoloration on the dorsum of the hands and forearms. Glossitis, either diffuse or localized, was present in every patient and was accompanied by atrophy of the papillae. In some patients imprints of the teeth could be seen along the margins of the tongue and in some individuals the tongue showed hemorrhagic suffusions on the inferior aspect. Cheilosis was present in two patients (Cases 1 and 8). In every subject the hair was dry and brittle.

Numbness and tingling of the hands and feet were complaints common to all patients. Four of the patients complained of dizziness when standing. Other abnormal neurologic signs were found in only two of the subjects. One (Case 2) had pain on pressure of the muscles of the legs. She also complained of sciatic pain. These symptoms were relieved with thiamine. Another (Case 3) presented a flaccid paraplegia, and the tendon reflexes were absent. She had a history of syphilis with positive serology as determined by Kahn and Meinicke

tests. The spinal fluid examination showed a syphilitic gold curve and an increase in globulin content.

Seven patients had dependent pitting edema. The five patients above 50 years of age had arteriosclerosis of varying degree of severity. Abdominal examinations showed meteorism, slight distention, and softness to palpation in all cases. In one patient (Case 3) there was ascites. The liver and spleen were not enlarged in any of the patients. Aside from emphysema in two subjects, there were no abnormal thoracic findings. Rectoscopic examinations were performed in six subjects. The mucosa of the rectum showed variable degrees of atrophy, paleness, and slickness.

TABLE I. BLOOD INDICES

CASE	PATIENT	MEAN CORP. VOL. (CU. MICRONS)	MEAN CORP. HB. (MICRO MICROGRAMS)	MEAN CORP. HB. CONC. (%)
1	C. G.	128	45	38
2	M. S.	105	36	34
3	C. B.	145	46	32
4	J. C. L.	134	44	30
5	G. A.	117	44	38
6	A. M.	131	47	36
7	A. F.	130	44	34
8	P. E.	119	42	32
9	J. G.	142	49	35

All the patients had macrocytic anemia with mean corpuscular volumes of from 105 to 145 cubic microns, mean corpuscular hemoglobins of from 34 to 47 micro micrograms, and mean corpuscular hemoglobin concentrations of from 30 to 38 per cent (Table I). The erythrocyte counts ranged from 1.47 to 2.28 million per cubic millimeter. The red blood cells showed marked anisocytosis, poikilocytosis, and polychromasia. Five subjects (Cases 2, 4, 5, 7, and 9) had leucocyte counts below 5,000 per cubic millimeter but only one (Case 5) had a count less than 3,000. The hemoglobin values ranged from 5.6 to 10.1 Gm. per 100 c.c., and the initial reticulocyte counts were less than 2.7 per cent. Bone marrow differentials showed the erythroblastic arrest characteristic of pernicious anemia, but in general there was only a 3 to 2 or a 2 to 1 predominance of myeloid over erythroid elements (Table II). In one patient only (Case 5) was there a 1 to 1 distribution of nucleated red cells to white cells.

Gastric analyses showed none of the cases to be histamine refractory, but, on the contrary, the patients were found to have normal or increased gastric acidity (Table III).

TABLE III. GASTRIC ANALYSES

CASE	PATIENT	FASTING		AFTER HISTAMINE	
		FREE°	TOTAL°	FREE°	TOTAL°
1	C. G.	60	70	65	80
2	M. S.	60	70	50	60
3	C. B.	0	10	40	50
4	J. C. L.	0	10	30	40
5	G. A.	0	12	90	100
6	A. M.	40	50	95	100
7	A. F.	0	15	45	50
8	P. E.	15	20	65	75
9	J. G.	30	45	115	130

TABLE II. DIFFERENTIAL BONE MARROW COUNTS

CASE	PATIENT	BASOPHILES (PER CENT)	EOSINOPHILES (PER CENT)	MYELOCYTES (PER CENT)	METAMYELOCYTES (PER CENT)	BAND (PER CENT)	SEGMENTED (PER CENT)	LYMPHOCYTES (PER CENT)	CLASMATOCYTES (PER CENT)	MEGAKARYOCYTES (PER CENT)	PLASMA CELLS (PER CENT)	RETICULUM CELLS (PER CENT)	NORMOBLASTS (PER 100 W.B.C.)	LATE ERYTHROBLASTS (PER 100 W.B.C.)	EARLY ERYTHROBLASTS (PER 100 W.B.C.)	MEGALOBLASTS (PER 100 W.B.C.)	NUMBER OF NUCLEATED R.B.C./100 W.B.C.
1	C. G.	0	3	C-27 C-22	20	19	18	8	3	1	1	0	6	19	15	2	42
2	M. S.	0	3	B- 2 C-26.5	18	10	10	33	1	0	1	0	25	12	8	2	47
3	C. B.	0	2.5	B- 1 C-17	15.5	12.5	23.5	16	1.5	1	0	0	15	18	16	5	54
4	J. C. L.	0	2.5	B- 0.5 C-37	17	7.5	32.5	20.5	0.5	0	0	2	28	24.5	29.0	15.5	97
5	G. A.	0	1	B- 2 C-13.5	28	7	9	14	3	1	0	0	23	10	16	11	60
6	A. M.	0	6	B- 1.5	17	5	30	23.5	3.5	0	0	0	12	35	17	3	67
7	A. F.	0	1	C-27 C-17	28	11	10	20	0	1	1	0	12	22	7	0	41
8	P. E.	0	3	B- 3	17	17	26	13	1	1	2	0	4	12	15	4	40
9	J. G.	0	2	C-10	10	10	49	17	1	1	0	0	34	17	4	4	59
							After Folic Acid Administration										
1	C. G.	0	1	C-25	12	14	25	17	5	1	0	0	71	13	3	0	87
8	P. E.	0	13	C-12 C-18	8	16	31	18	1	1	0	0	109	7	5	0	121
9	J. G.	0	1	B- 1	8	10	26	32	2	1	0	1	117	4	2	0	123

TABLE IV. BLOOD CHEMISTRY

CASE	PATIENT	SERUM PROTEINS				CHOLESTEROL		AMYLASE (UNITS PER 100 C.C.)	LIPASE (C.C. 1/20N NaOH PER 100 C.C.)	CALCIUM (MG./100 C.C.)	PHOSPHORUS (MG./100 C.C.)	POTASSIUM (MG./100 C.C.)
		TOTAL (GM./100 C.C.)	ALBUMIN (GM./100 C.C.)	GLOBULIN (GM./100 C.C.)	FIBRINOGEN (MG./100 C.C.)	TOTAL (MG./100 C.C.)	ESTERS (MG./100 C.C.)					
1	C. G.	4.2	2.4	1.8								
2	M. S.	4.0	2.5	1.5	600	306	169			11.5	3.0	16.0
3	C. B.	4.6	2.5	2.1	400	190	99	108	1.0	11.0	4.2	13.0
4	J. C. L.	5.0	2.8	2.2	400							
5	G. A.	3.3	1.9	1.4		285	148	109	2.0	11.5	3.5	13.5
6	A. M.	5.0	2.2	2.4	400	203	128	110	0.3	12.0	3.5	10.0
7	A. F.	3.6	1.9	1.7		230	120	100	2.0	12.0	4.0	16.0
8	P. E.	5.4	3.6	1.8		225	180	98	1.3	10.8	5.0	13.4
9	J. G.	5.5	3.1	2.4	400	187	110	102	0.7	12.0	4.0	16.0

In all patients tested, the blood calcium, phosphorus, potassium, amylase, and lipase values were normal (Table IV). The total cholesterol was normal or moderately increased, with the cholesterol ester ratio to total cholesterol moderately decreased. Serum proteins were as a rule low, from 3.3 to 5.4 Gm. In only two cases was the ratio of albumin to globulin over 1.5. In seven patients it was from 0.9 to 1.3. Serum iron determinations were performed in six instances. The values ranged from 83 to 247 micrograms of iron with an average of 156 micrograms. Only two values (197 and 247 micrograms) were above the normal range (from 50 to 180 micrograms).[6]

No gastroscopic examinations were performed in this series; however, in a similar series of twenty-five cases found in Cuba, Milanes reported that most of the patients had localized areas of atrophic and hypertrophic gastritis and a few had diffuse atrophic gastritis.[7]

A study of the feces showed that the reaction was acid in seven cases. In these the values for fatty acid determined by the method of Labbé and Larué were above 11 c.c. of 0.1 N sodium hydroxide. The amounts were well above the normal range (from 0 to 7 c.c. of 0.1 N sodium hydroxide). (Table V.) The special determinations of total fats, neutral fats, and other information will be reported separately. The starch residue was above normal. Bile pigments were present in normal amounts. The feces in four subjects (Cases 1, 3, 5 and 6) were infested with *Trichuris trichiura*. The feces of cases 3 and 7 contained ova of *Ascaris lumbricoides*. One subject (Case 1) also expelled one *Ascaris lumbricoides*, but several serial examinations were negative for ova. Another patient (Case 6) had *Giardia intestinalis* diagnosed in material obtained by duodenal intubation. The feces of this patient also contained ova of *Necator americanus*. In each case bacteriologic examination of the stools and of material obtained by curettage of rectal mucosa showed that pathogens were not present. Samples obtained from the small intestine through a Miller-Abbott tube yielded no pathogenic bacteria.

TABLE V. STOOL ANALYSES

CASE	PATIENT	pH	FATTY ACIDS c.c./0.1 N NaOH (PER 100 GM. MOIST FECES)	PATHOGENS AND PARASITES
1	C. G.	6.0	16.0	Ova of Trichuris trichiura, Ascaris lumbricoides
2	M. S.	4.5	12.0	None
3	C. B.	7.5	11.0	Ova of Trichuris trichiura; ova of Ascaris lumbricoides
4	J. C. L.	5.5	12.3	None
5	G. A.		14.2	Ova of Trichuris trichiura
6	A. M.	7.5	11.2	Ova of Trichuris trichiura, Necator americanus, Giardia intestinalis
7	A. F.	6.5	15.0	Ova of Ascaris lumbricoides
8	P. E.	4.5	12.3	None
9	J. G.	5.5	17.8	None

RESULTS FOLLOWING ADMINISTRATION OF FOLIC ACID

When all the preceding findings were obtained, each of the nine patients was given folic acid, orally administered in water suspension between meals. The folic acid was weighed on an analytic balance. Six subjects (Cases 1, 2, 5, 7, 8, and 9) were given 100 mg. twice daily. Subsequent experience having indicated that the daily dosage of 200 mg. was more than necessary, the daily dose was decreased to 100 mg. after the patient has passed the peak of his reticulocyte response. Three subjects (Cases 3, 4, and 6) received 10 mg. of folic acid per day. One subject (Case 2) received, in addition, 100 mg. of thiamine chloride daily, intramuscularly and another (Case 7) received, in addition to the folic acid, 100 Gm. of a yeast extract* each day; on one occasion the latter patient was given 250 c.c. of plasma intravenously to reduce the amount of edema present. Two laboratory workers, who had red counts of 5.15 and 5.24 millions per cubic millimeter and hemoglobins of 15.8 and 16.2 Gm., respectively, received 200 mg. each of folic acid, daily for ten days, and served as controls.

The response of the patients receiving 10 mg. of folic acid has so closely paralleled the response of those receiving 200 mg. that they will not be discussed separately. With the exception of one subject (Case 2) who had a deficiency of both niacin and thiamine, and another (Case 7) who had anasarca, all the patients began to recover strength and to experience a feeling of well-being within three or four days after the initiation of folic acid therapy. The response of these two patients to folic acid was similar but perhaps retarded somewhat. In all cases the pigmentation of the skin decreased. In one patient (Case 9) who has been treated with folic acid for forty-one days the skin has cleared almost entirely. The patients have not been outside the hospital since their admission, so that this may be due in part to lack of sunshine. In every subject there was a striking change in the facial expression, which became alert and lively, whereas prior to treatment, it had been almost masklike. The con-

*Basamin, Anheuser-Busch, St. Louis, Mo.

junctival mucosa changed from intense paleness to varying degrees of recoloration which was in direct proportion to the increase in blood hemoglobin and erythrocyte values.

Three representative cases are reported briefly; they give the reader an idea of the striking improvement effected by folic acid.

CASE 4.—J. C. L., a 63-year-old white man, was admitted to the Calixto Garcia Hospital Nov. 19, 1945, complaining of diarrhea, weakness, and dizziness.

Throughout his adult life his diet had consisted chiefly of root vegetables and corn meal. Occasionally he ate an orange or a piece of pineapple and once or twice a month he ate a piece of beef. Although he noticed that he was losing strength some years ago, he dates the onset of his present illness to seven months before admission to the hospital. At this time he noticed burning and soreness of the tongue and small sores in the mouth. Almost simultaneously he began to pass very large bulky yellow or white stools. His appetite became so poor and he became so weak that he consulted his physician in Cienfuegos, who referred him to us for treatment.

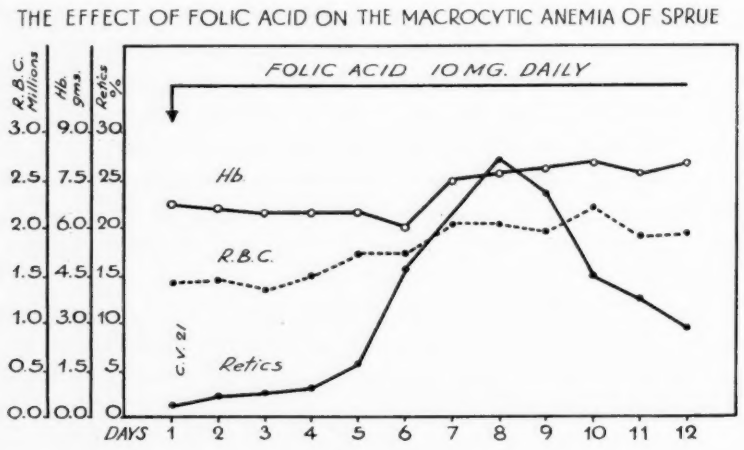

Fig. 1.

Physical examination showed an extremely emaciated, pale, apprehensive man. By the time he came to us for treatment we had learned that the administration of 10 mg. of folic acid daily by mouth was followed by a positive response. Accordingly, he was given this dosage. His hemopoietic response is shown in Fig. 1. Within ten days after treatment was initiated, the profuse liquid, light-colored stools became normal in color; they were solid and much smaller in volume. Since then, the stools have been copious and semisolid on some days, and other days they have been well formed and small in volume. His appetite at the present time is tremendous. He has no alimentary tract symptoms, his strength has returned, and he is eager to return to work.

CASE 5.—G. A., a 62-year-old man, was admitted to the Calixto Garcia Hospital Nov. 22, 1945, complaining of diarrhea, burning of the esophagus, and asthenia.

He considered that his health had been perfect until the onset of the present illness four months before admission to the hospital, despite the fact that during all his adult life his diet had consisted chiefly of rice, potatoes, corn meal, root vegetables, avocados, and occasionally a glass of milk, an egg, or a small serving of pork. The onset of his illness was sudden and was preceded only by "indigestion" and gastric distress which, within a

few days, was followed by loss of appetite and severe diarrhea. He had from eighteen to twenty foul-smelling stools daily which were frothy and sometimes yellow in color and sometimes "like clear water." Although he passed no blood, he sometimes had severe burning of the rectum. A few days after the onset of his illness he became so weak and dizzy that it interfered with his work. During the four months he was ill he lost thirty-eight pounds in weight.

Physical examination showed a small, pale, emaciated man. The buccal mucosa was extremely pale as was the tongue except where it was bright red at the borders and tip. The papillae were smooth and the tongue was edematous at the border so that the imprint of his teeth was clearly visible. Dependent pitting edema over the dorsum of the feet extended halfway up the legs and made it impossible for him to raise his feet or put on his shoes.

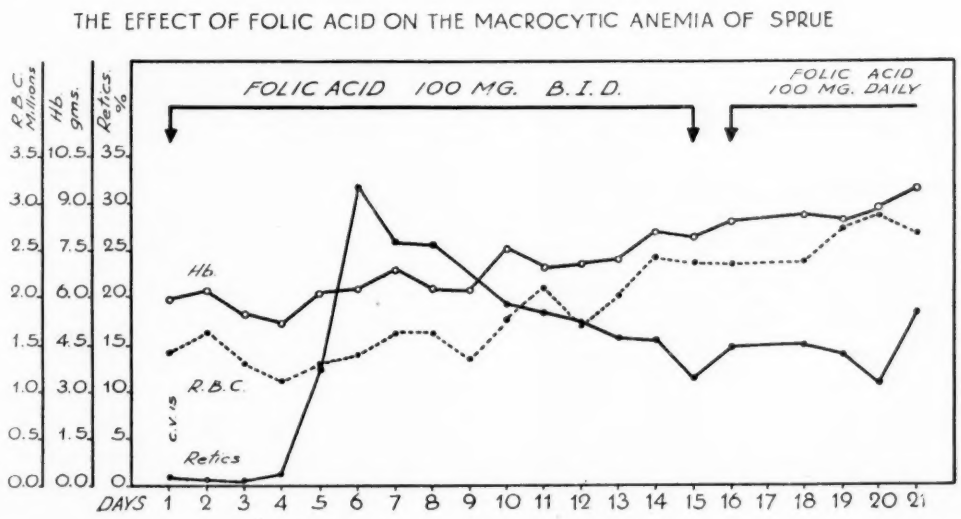
THE EFFECT OF FOLIC ACID ON THE MACROCYTIC ANEMIA OF SPRUE
R.B.C. Millions
Hb. gms.
Retics. %
FOLIC ACID 100 MG. B.I.D.
FOLIC ACID 100 MG. DAILY
Hb.
R.B.C.
C.V. 1.5
Retics
DAYS 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Fig. 2.

He was given 100 mg. of folic acid twice daily by mouth. Two days later his appetite became tremendous and the soreness of the tongue disappeared. Soon after this the stools became semisolid and on some days were well formed and normal in color. Frequently he had only one or two stools daily and often they amounted to only 100 c.c. in volume. The edema has disappeared, and he says that he feels stronger than he has felt for years and that he wishes to return to work. His hemopoietic response to treatment is shown in Fig. 2.

CASE 8.—P. E., a 63-year-old man, was admitted to the Calixto Garcia Hospital Nov. 19, 1945, complaining of diarrhea and weakness.

During most of his adult life he had been unable to eat pork or beef because they caused gastric distress. He rarely drank milk. For a number of years his diet had consisted chiefly of potatoes, root vegetables, corn in various forms, and from three to four eggs daily. Other than the gastric distress following the ingestion of beef and pork, his health had been perfect until five years before admission to the hospital, when he noticed a burning sensation of the mouth and tongue and swelling of the tongue. Soon after this he began having severe diarrhea. Sometimes he had thirty bowel movements daily which were usually preceded by severe colic. The stools were yellow in color and foamy. He consulted a physician who gave him liver extract which he took intermittently for three years, during which time he remained well. A year and one-half after he discontinued taking liver extract, he again began having diarrhea, and all the symptoms he had during his first illness reappeared. He

lost his appetite, began having paresthesias of the legs, dizziness, and tinnitus and became progressively weaker. After losing twenty pounds in weight he reported to his physician, who sent him to us.

Physical examination showed a well-developed, pale, emaciated man who lay quietly in bed with no apparent desire to move. The tongue was slick and shiny and was intensely red along the tip and edges.

He was given 100 mg. of folic acid twice daily by mouth. Three days after this therapy was initiated, his appetite, strength, and vigor greatly increased. Prior to treatment the volume of the stools had been about 1,000 c.c. daily, and they had been light in color and frothy (see Figs. 3 and 4). The volume gradually decreased and the color became more normal. Although the stools have not become completely normal, he says he feels stronger than he has felt for many years and that he wishes to return to work. His hemopoietic response to folic acid is shown in Table VI (Case 8).

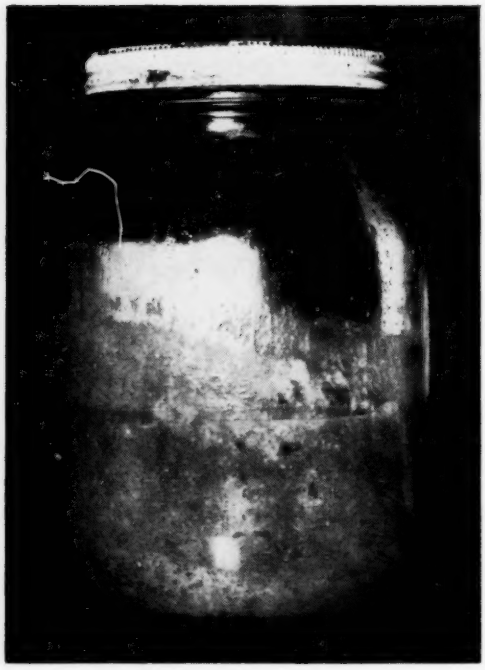

Fig. 3.

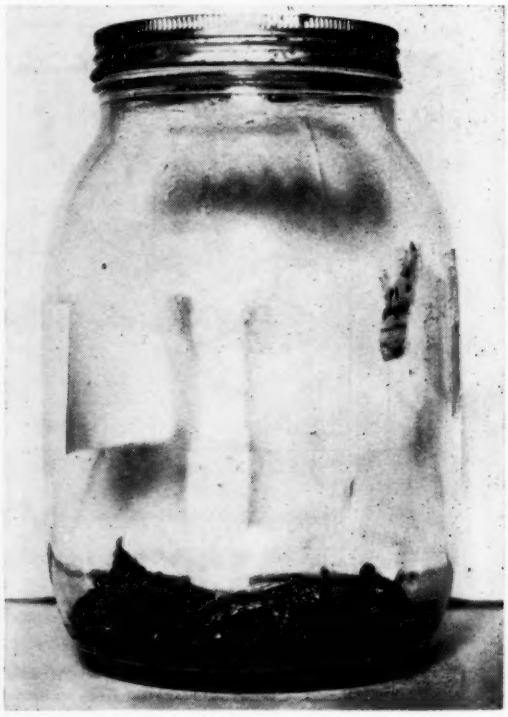

Fig. 4.

Fig. 3.—Photograph taken before folic acid therapy. Note consistency and copious amount of the stools.

Fig. 4.—Photograph taken ten days after folic acid therapy was begun. The amount of fecal material has decreased greatly, the stool is no longer liquid, and it is more nearly normal in color.

The hematologic data are presented in Table VI. Following therapy with folic acid, reticulocytosis occurred in every instance with peak values of from 12.5 to 31.8 per cent on 200 mg. daily, and from 18.8 to 26.2 per cent on the 10 mg. dosage given to three of the patients (Cases 3, 4, and 6). In all cases the response was maximal according to the Minot and Castle formula based on the initial erythrocyte count.[8] The day of the peak ranged from the sixth

TABLE VI. ANTIANEMIC EFFECT OF FOLIC ACID IN SPRUE

CASE	PATIENT	RED CELLS (MILLIONS/C.MM.)			HEMOGLOBIN (GM./100 C.C.)			RETICULOCYTOSIS			FOLIC ACID (MG. PER DAY)	NUMBER OF DAYS OF ADMINISTRATION
		INITIAL	14 DAYS	FINAL (DAY)	INITIAL	14 DAYS	FINAL (DAY)	INITIAL %	DAY OF PEAK	% AT PEAK		
1	C. G.	1.47	2.55	3.82 (37)	6.8	8.6	11.9 (37)	1.4	6	22.7	200 100	22 15
2	M. S.	1.99	2.60	2.96 (32)	7.2	10.0	10.8 (32)	2.6	6	19.5	200 100	15 17
3	C. B.	1.52	2.40	2.40 (14)	7.1	10.7	10.7 (14)	1.6	8	22.6	10	14
4	J. C. L.	1.57	2.03	3.20 (23)	7.0	7.4	10.7 (23)	1.1	7	26.2	10	23
5	G. A.	1.28	2.49	3.24 (32)	5.6	8.1	10.7 (32)	0.8	6	31.8	200 100	15 17
6	A. M.	2.13	2.84	3.45 (18)	10.1	11.7	12.4 (18)	0.8	8	18.8	10	18
7	A. F.	2.16	2.66	3.05 (41)	9.5	9.7	11.3 (41)	1.6	7	17.2	200 100	24 17
8	P. E.	2.28	2.85	3.93 (31)	8.3	10.0	11.0 (31)	1.1	8	12.5	200 100	15 16
9	J. G.	1.97	3.04	3.94 (41)	9.7	10.4	13.8 (41)	1.9	7	17.2	200 100	25 16

to the ninth. The red cell counts rose from 0.50 to 1.21 million per cubic millimeter in fourteen days in the patients receiving 200 mg. of folic acid. It increased from 0.46 to 0.71 million per cubic millimeter in the patients receiving 10 mg. doses. (See figures in representative case histories.) One subject (Case 5) was given 200 mg. of folic acid each day for fifteen days and 100 mg. daily for seventeen days. He had a reticulocyte response of 31.8 per cent on the sixth day. Another (Case 4, Fig. 1) was given 10 mg. of folic acid each day. His initial erythrocyte count was 1.57 million per cubic millimeter. His reticulocytes rose to 26.2 per cent on the seventh day of treatment. His red cell count and hemoglobin values increased steadily even at this lowered level of administration. The patients who have been under treatment for from thirty-two to forty-one days have had erythrocyte increases from 1.05 million (Case 2) to 1.97 million per cubic millimeter (Case 9). One (Case 2) had a low-grade fever caused by abscessed teeth. Later in the study, dental extractions were performed, and her temperature thereafter was normal. Her erythrocyte count rose 1,000,000 per cubic millimeter in the following nine days. The hemoglobin values of all the cases increased as the erythrocyte count increased. The leucopenia generally accompanying macrocytic anemia was decreased by the administration of folic acid. In some cases the white counts rose as high as 20,000 per cubic millimeter and in other cases it increased to normal levels. The elevations did not persist, but white counts remained from 5,000 to 9,000 per cubic millimeter.

The normal control subjects who received 200 mg. of folic acid orally for ten days did not show any changes in their erythrocyte counts, hemoglobin, leucocyte counts, or number of reticulocytes in the four weeks following its administration.

In four of the patients who have been treated with folic acid for more than thirty days, the mean corpuscular volumes have decreased as follows: Case 1, from 122 to 94 cubic microns; Case 7, from 130 to 114; Case 8, from 119 to 94; and Case 9, from 145 to 109.

Bone marrow studies were repeated in three patients (Cases 1, 8, and 9) who had been treated for thirty-seven, thirty-one, and forty-one days, respectively. Macroscopically, there was a gross change from light red, thick, gelatinous marrow to deep red fluid containing discrete clumps of cells. Microscopically, the erythroblastic arrest was substituted by a normoblastic hyperplastic regeneration. There was a shift in ratio of white cells to nucleated red cells of from 5 to 2 to from 5 to 6 (Table II). The findings in the bone marrow from the other patients after treatment will be reported later since these subjects are still under treatment and the reticulocytes are still high.

The appetite improved rapidly soon after medication was started. Those who were under treatment for long periods of time (from thirty to forty days) have tremendous appetites and often request additional servings. In every instance, the glossitis has faded, and in fine subjects (Cases 1, 2, 4, 5, and 9) the tongue has become entirely normal. The soreness of the tongue and mouth has totally disappeared, and patients can now eat and smoke without any sensation of burning of the tongue or mouth. The rectal burning about which some patients complained has also disappeared.

The paresthesias of the hands and feet have not yet been affected by the administration of folic acid. Edema disappeared in all patients with the exception of two (Cases 3 and 9). One subject (Case 3) had anasarca and nervous manifestations that might have been due to thiamine deficiency; she has been under treatment only fourteen days. Another subject (Case 9) had a reappearance of discrete ankle edema. At the time this report was submitted, the abdominal distention had not disappeared, and the patients still complained of "gas."

The diarrhea followed a variable course. It gradually decreased in most cases. At present, soft brown stools tending toward normal have replaced the original typical stools seen in sprue in four patients (Cases 3, 4, 5, and 6). The number of stools has decreased from three to fourteen to from one or two daily. At present the stools of one subject (Case 2) have returned to normal, but this patient had, in addition to folic acid, supplementary nicotinic acid therapy. Two subjects (Cases 8 and 9) still pass the same spruiform type of stools as they did when they were admitted, although for the last two days the stools of one of them (Case 8) have been soft and brown. The fact that there is such variation in the stools from normal to pathologic, and vice versa, leads us to the conclusion that at present no dogmatic statement can be made concerning the efficacy of folic acid alone in controlling the diarrheal aspect of sprue. Those subjects showing persistent improvement of the diarrhea with no tendency toward relapse to the spruiform type are the ones in whom the illness is of more recent onset. In these patients the course of therapy has been more effective in controlling the diarrhea than it has been in the chronic

cases. In all patients, shortly after the beginning of treatment, there was an accompanying physical improvement and sense of well-being. There was a tendency for the stools to become well formed, more normal in color, and much less in amount. For several days the stools would appear practically normal but this period would be followed for a day or so by copious, frothy stools. We are still studying the effect of folic acid on the alimentary tract and specifically upon the appearance of the stools. It suffices to say at this time that there is a great change in the majority of instances toward the normal following administration of folic acid.

DISCUSSION

The present findings show that synthetic folic acid produces great improvement in patients with tropical sprue in relapse. These, as well as previous observations by one of us (T. D. S.) and associates, demonstrate that folic acid has a striking antianemic effect in nutritional macrocytic anemia and sprue. Moore, Bierbaum, Welch, and Wright[9] have confirmed these observations on the antianemic effect in pernicious anemia and have reported that folic acid has proved effective in one case of nontropical sprue and one case of pernicious anemia of pregnancy. Little is understood about the pathogenesis of these macrocytic anemias, and it is possible that these studies will open a new era in effecting a better understanding of the physiology of the blood-forming organs. Until more information is available, we suggest that patients who have achylia following histamine injection be considered tentatively to have Addisonian pernicious anemia and that, in cases with proved steatorrhea and macrocytic anemia, sprue be considered as a tentative diagnosis. Our criteria for the diagnosis of nutritional macrocytic anemia have been described fully in previous publications, and, after further intensive investigations, we see no reason to change our method of making this clinical diagnosis.[5] We now suggest, however, that the so-called macrocytic anemia of pellagra and the pernicious anemia of pregnancy, which have been found to respond to folic acid, be considered as forms of nutritional macrocytic anemia and classified as such.

The physician must always make every effort to prove each diagnosis. Because it is often difficult to grasp the fundamental mechanism behind the disease, in many cases the clinical diagnosis is arbitrary. Certainly more studies are needed.

Despite the fact that pernicious anemia is included in the group of macrocytic anemias, it is obvious that in its natural pathogenesis it is somewhat different from the macrocytic anemia of sprue and from nutritional macrocytic anemia. One of us (T. D. S.) has the working hypothesis that folic acid in many of the foods occurs as a conjugate and that, in view of Castle's work, it is likely that in pernicious anemia the enzymes are unable to liberate the folic acid efficiently whereas in sprue, pellagra, pregnancy, and nutritional macrocytic anemia, the folic acid or substances acting similarly are more available.

Some explanation must be made concerning the fact that fairly large amounts of folic acid are required to produce a maximum hemopoietic response in contrast to potent liver extract in which the active substance in the thera-

peutic dose is apparently smaller. (One of us, T. D. S., has observed three patients who did not respond significantly on 5 mg. of folic acid but who did respond fairly satisfactorily on 10 mg. per day administered by mouth.) Unpublished observations by one of us (T. D. S.) show that a concentrate of dried brewer's yeast* produces a satisfactory hemopoietic response when 100 Gm. of the material is given by mouth daily. This amount of material contains approximately 1 mg. of folic acid. An explanation of this is probably similar to that previously described for the liver extract.

Since the clinical improvement that follows the administration of folic acid parallels that which follows the administration of potent liver extract, one might expect that folic acid in adequate doses would be as effective as liver extract in treating sprue. That folic acid is an effective hemopoietic agent is certain, but it probably will not have a curative effect above and beyond that afforded by large doses of a potent liver extract.

It seems likely that the natural pathogenesis of sprue and nutritional macrocytic anemia is related to an inadequate diet. We have found that animal protein or yeast and the nutrients included are effective in the treatment and prevention of this type of anemia. Yet, despite the fact that the patients in this study were restricted to a diet deficient in protein throughout the study, they had a striking clinical and hemopoietic improvement. (We recommend a high vitamin, high protein diet in the treatment of sprue, as folic acid cannot be expected to substitute for an adequate diet.)

Many of these patients were infested with intestinal parasites and they improved without any antiparasitic therapy, a point which will be more fully discussed in subsequent papers.

These studies on tropical sprue support the practical point of view that folic acid has a striking antianemic effect in some types of macrocytic anemias in relapse.

SUMMARY AND CONCLUSIONS

The present study has shown that synthetic folic acid produced great improvement in nine patients with tropical sprue in relapse. The subjects all had a good gain of body weight, they gained in strength, and the glossitis disappeared. The stools in all cases tended to become much nearer normal and in some instances they appeared normal. Studies directed toward obtaining a better understanding of the function of the alimentary tract are now under way. These studies and the previous ones by one of us (T. D. S.) and associates demonstrate conclusively that synthetic folic acid has a striking antianemic effect on persons with sprue, pernicious anemia, and nutritional macrocytic anemia and open a new era of better understanding of the physiology of blood formation. Despite the fact that pernicious anemia is included in the group of macrocytic anemias responding to folic acid therapeutically, it is obvious that its natural occurrence is different from that of sprue and of nutritional macrocytic anemia. Folic acid should be considered an antianemic substance, and our observations would suggest that the response to liver extract and

*Basamin, Anheuser-Busch, St. Louis, Mo.

to dried brewer's yeast powder is out of proportion to the amount of folic acid which they contain. It is truly remarkable that the patients improved so much from a clinical and laboratory point of view while restricted to a diet devoid of meat and meat products. We do not recommend such a restriction in diet in the day-to-day treatment of sprue. In fact, we stress a high vitamin, high protein diet as folic acid cannot be expected to substitute for an adequate diet.

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LABORATORY METHODS

A METHOD FOR DETERMINING THE RELATIVE ANTICONVULSIVE ACTIVITY OF BARBITURATES

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PREVIOUSLY one of us demonstrated the prolonged activity of pentobarbital and phenobarbital in antagonizing the convulsant action of either strychnine or picrotoxin.[1] It was suggested that this work might serve as the basis of a method for determining the comparative length of action of barbiturates. Although further studies indicated that this antagonism does not yield a true indication of the length of action of barbiturates relative to sedation or anesthesia, some information was obtained on the comparative protection offered by the barbiturates against convulsants and the duration of such protection. It was thought that this information might serve a useful function for some types of work. Although both picrotoxin and strychnine were used to produce death by convulsions, only picrotoxin proved satisfactory.

PROCEDURE

In these experiments male rats of a uniform strain (Purdue), source, and weight from (66 to 80 grams) were used, unless otherwise indicated. The animals were fed a commercial ration* ad libitum. At least ten rats were used for each run. When more than ten rats were used, it was to confirm a previous result. Since in each instance there was no significant difference, the data indicate that ten rats of this strain and of the same sex and weight were adequate. Rats from another source were tried in which ten rats of the same sex and weight were not adequate for consistent results.

All injections, 1 c.c. per 100 grams of body weight, were made intraperitoneally. Sodium salts of the barbiturates were used when available. When they were not available, the barbiturates were put into solution with the least possible amount of normal sodium hydroxide. The dose of strychnine sulfate was 3 mg. per kilogram. This dosage regularly killed seven of ten rats in the weight range used. The dose of picrotoxin was 10.5 mg. per kilogram. This dose killed nine of ten rats in the weight range of from 66 to 80 grams and seven of ten rats in the weight range of from 82 to 104 grams. The rats were weighed before the barbiturate was given and reweighed before the convulsant when the interval was longer than two hours. In such cases the second weight was always taken as the basis for the dose of convulsant. The temperature of the room where the experiments were done was kept at from 77 to 81° F. to rule out the effect of variations in temperature.[2] The rats were placed in this room at least twenty hours before an experiment was started.

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*Purina dog chow checkers.

A protective dose of barbiturate was determined. This was done by giving doses of barbiturate increased by increments of 20 per cent until not more than one of ten rats died from the convulsant given at the standard interval after the barbiturate. A twenty-minute standard was used for those compounds too short in action for a feasible dose of barbiturate to be given one hour before the convulsant. The number of deaths caused by the convulsant, when given at longer intervals after the barbiturate, was then compared with the number of deaths determined at the standard interval.

RESULTS AND DISCUSSION

As may be seen from Table I, several barbiturates required a fatal dose to be effective against strychnine. Of the nine compounds listed, only sodium phenobarbital did not produce any deaths at the dose level required. At a dosage greater than the $L.D._{50}$, sodium ortal failed to protect the survivors against strychnine. Data obtained from these studies with strychnine are presented in Table II. With sandoptal, the protective action was gone in two hours. Sodium amytal, sodium pentobarbital, and sodium n-butyl allyl bar-

TABLE I. EFFECT OF SUFFICIENT BARBITURATE TO REDUCE DEATHS FROM STRYCHNINE SULFATE* TO 10 PER CENT OR LESS

BARBITURATE	DOSE (MG./KG.)	NUMBER OF RATS USED	NUMBER OF RATS DEAD FROM BARBITURATES	PER CENT OF RATS DEAD FROM BARBITURATES
Na phenobarbital	75	120	0	0
Na amytal	75	34	1	3
Neonal	62.5	45	4	9
Sandoptal	75	28	5	18
n-butyl allyl Na barbiturate	80	27	6	22
Na pentobarbital	40	26	6	23
Dial	100	79	29	37
Na barbital	300	213	84	39
Na alurate	90	67	32	48

*The dose of strychnine sulfate was 3 mg. per kilogram. This killed 70 per cent of untreated rats.

TABLE II. BARBITURATES LISTED IN ORDER OF INCREASING LENGTH OF ACTION AGAINST STRYCHNINE

		TIME AFTER BARBITURATE—3 MG./KG. STRYCHNINE SULFATE GIVEN							
		ONE HOUR		TWO HOURS		SIX HOURS		FORTY-EIGHT HOURS	
BARBITURATE	DOSE (MG./KG.)	NUMBER OF RATS USED	NUMBER OF RATS DEAD	NUMBER OF RATS USED	NUMBER OF RATS DEAD	NUMBER OF RATS USED	NUMBER OF RATS DEAD	NUMBER OF RATS USED	NUMBER OF RATS DEAD
Sandoptal	75	10	0	10	9				
Na amytal	75	10	0	10	5				
Na pentobarbital	40	10	0	10	5				
n-butyl allyl Na barbiturate	80	10	0	10	5				
Dial	100	10	1	10	2	20	13	10	8
Neonal	62.5	10	0	10	2	10	3	10	9
Na alurate	90	10	0	10	1			10	4
Na barbital	300	10	1	10	0	10	1	30	11
Na phenobarbital	75	10	0	10	1	20	3	20	3

biturate exerted very little, if any, protective activity two hours after administration. Dial showed protection at two hours but not at six hours. Neonal showed protection at six hours but not at forty-eight hours after its administration. Sodium alurate, sodium barbital, and sodium phenobarbital gave evidence of protection forty-eight hours after administration. The grouping of these compounds was much the same as that derived from other methods of determination of length of action.[3, 4] However, strychnine as a test substance was abandoned because it (1) failed to give real additional information, (2) required too many animals, and (3) required the use of animals resistant to the barbiturate.

TABLE III. BARBITURATES SHOWING INTERMEDIATE AND PROLONGED PROTECTION AGAINST PICROTOXIN

BARBITURATE	DOSE (MG./KG.)	TIME AFTER A BARBITURATE—10.5 MG./KG. OF PICROTOXIN WAS GIVEN											
		ONE HOUR		TWO HOURS		FORTY-EIGHT HOURS		SEVENTY-TWO HOURS		NINETY-SIX HOURS		ONE HUNDRED TWENTY HOURS	
		NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD
Na ortal	110.0	10	1	10	8	10	6						
Na amytal	30.0	10	1	10	7	10	8						
Na pentobarbital	16.0	10	0	30	20	30	4	10	3	10	7		
Neonal	13.6	10	0	20	11	10	5	10	6				
Na alurate	30.0	10	1	10	3	10	6	10	6				
Na barbital	60.0	10	0	10	1	10	2	10	2	10	6	10	6
Na ipral	20.0	10	0	10	1	10	0	10	0	10	5	10	8
Na phenobarbital	37.5	10	1	10	1	10	0	10	1	10	3	10	8

When picrotoxin was used as the convulsant agent, the results indicated that supplemental information, not obtained by other methods commonly in use, was obtained. For convenience in discussion, the eight barbiturates whose standard protection against picrotoxin was determined at one hour were divided into two groups: (1) those in which picrotoxin at two hours killed more than 50 per cent, and (2) those in which picrotoxin killed less than 50 per cent in a similar period (Table III and Fig. 1). Of the four compounds which gave less than 50 per cent protection at two hours, the sodium salts of amytal and ortal gave results not significantly different from each other. Neonal showed the same type of result, but the maximum death rate was not reached as quickly. Sodium pentobarbital showed a different type of result; whereas at two hours, 67 per cent were killed, at forty-eight hours only 13 per cent were killed. At seventy-two hours, 30 per cent were killed and at ninety-six hours 70 per cent were killed. The chance that the difference between the forty-eight hour value and the two-hour value was not significant was less than 1 in 100,000. The chance that the difference between the two-hour value and the seventy-two hour value was not significant was less than 1 in 100. Extra rats were run at these intervals as can be seen from Table III. There was no difference in results between the different runs. At present there is no explanation for this increase of protective action after the partial disappearance of protection.

Some studies on another group of rats indicated a similar type of protection, but the timing was different for the other strain of rats.

Of those compounds which showed less than 50 per cent deaths at two hours, the action of sodium alurate was the most brief. The protection seemed to be gone at forty-eight hours. There was no real difference between sodium barbital and sodium ipral. They both showed significant protection at seventy-

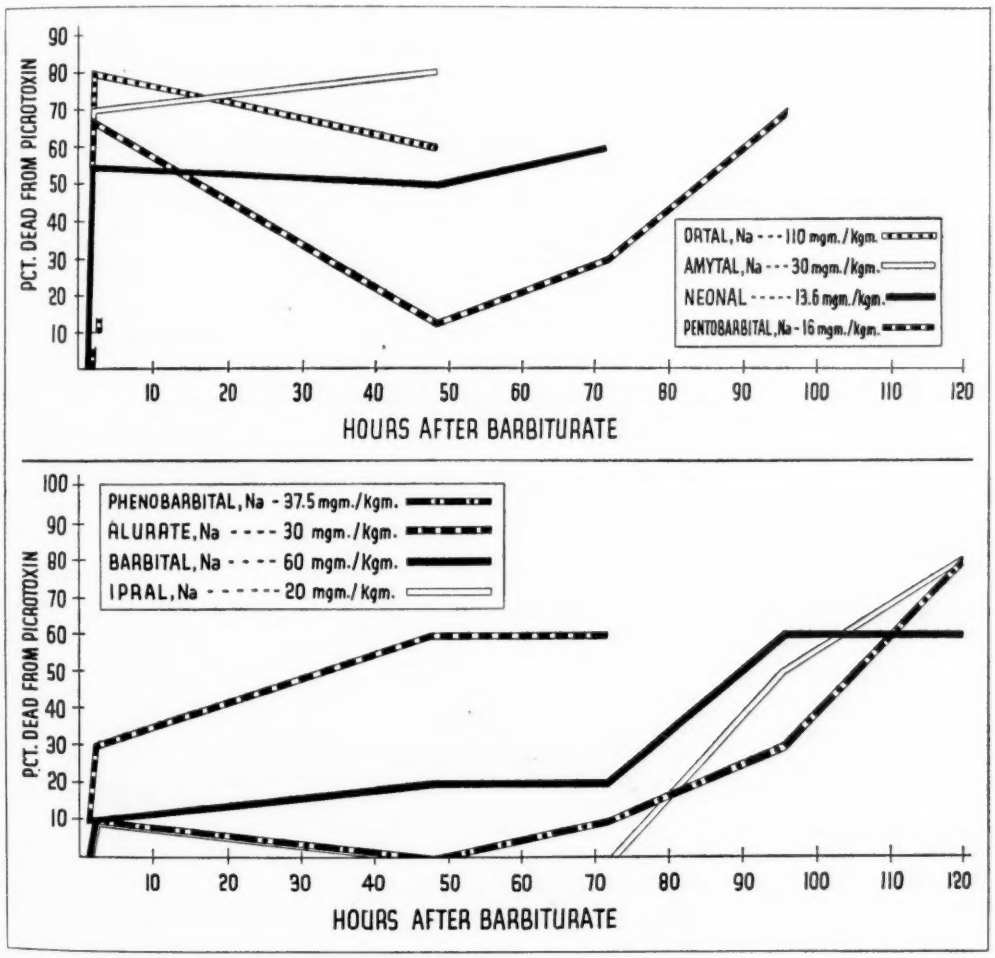
PCT. DEAD FROM PICROTOXIN
HOURS AFTER BARBITURATE
ORTAL, Na --- 110 mgm./Kgm.
AMYTAL, Na --- 30 mgm./Kgm.
NEONAL ----- 13.6 mgm./Kgm.
PENTOBARBITAL, Na - 16 mgm./Kgm.
PHENOBARBITAL, Na - 37.5 mgm./Kgm.
ALURATE, Na ---- 30 mgm./Kgm.
BARBITAL, Na ---- 60 mgm./Kgm.
IPRAL, Na ------ 20 mgm./Kgm.

Fig. 1.—Deaths from picrotoxin after barbiturates with medium and long anticonvulsive activity.

two hours but not at ninety-six hours. Sodium phenobarbital apparently showed some protection at ninety-six hours, but at 120 hours the effect of all barbiturates was worn off. Therefore, it may be assumed that ninety-six hours would be sufficiently long to run the test for real differentiation. Of the eight compounds which were studied with the standard at one hour, sodium ortal was the only one which required an anesthetic dose. This dose

TABLE IV. BARBITURATES SHOWING SHORT PROTECTION AGAINST PICROTOXIN

BARBITURATE	DOSE (MG./KG.)	TIME AFTER A BARBITURATE—10.5 MG./KG. OF PICROTOXIN WAS GIVEN									
		TWENTY MINUTES		FORTY MINUTES		SIXTY MINUTES		EIGHTY MINUTES		TWO HOURS	
		NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD	NUMBER RATS USED	NUMBER RATS DEAD
Na seconal	18.0	10	0	10	5	10	10	10	10	10	10
Sandoptal	25.0	10	0	10	4	10	7	10	9	10	9
Na pentothal	15.0	30	2	30	8	30	13	30	6	30	20
Na ortal	90.0	10	1	10	0	10	3	10	7	10	10

killed about one in thirty rats. For this reason sodium ortal was also tested with a standard interval of twenty minutes.

For the shorter acting compounds, the standard was determined at twenty minutes after the barbiturates (Table IV and Fig. 2). The dose of sodium ortal for this interval was not lethal but was anesthetic. Sandoptal was tried in doses up to the dose used against strychnine at one hour. Since sandoptal was still not effective against picrotoxin, that compound was tested at twenty

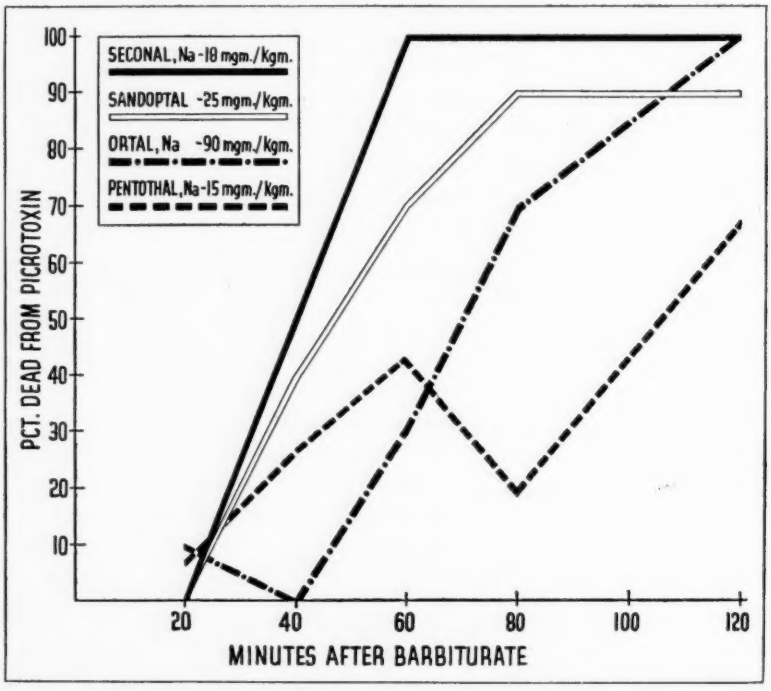

Fig. 2.—Deaths from picrotoxin after barbiturates with short anticonvulsive activity.

minutes. Here a dose just approaching the anesthetic level was necessary. Sandoptal was the only barbiturate in which the protective dose against picrotoxin was not smaller than the protective dose against strychnine for the one-hour standard. It can be seen that the protective action of sandoptal

was very short. It might be that since picrotoxin took longer to produce convulsions and kill than strychnine, most of the activity was gone by the time picrotoxin became effective; whereas, that was not true of strychnine. Of the four compounds studied with a twenty-minute standard for picrotoxin, sodium seconal, sandoptal, and sodium ortal all showed consistent decrease in activity with time. The activity of sodium seconal decreased most rapidly and that of sodium ortal least rapidly. Sodium pentothal showed a consistent decrease through the sixty-minute interval and then apparently an increase in activity at eighty minutes. The chance that the difference between these two times was not significant is about 1 in 20. It would appear that pentothal showed the same phenomenon as pentobarbital but not in so striking a manner.

SUMMARY

Strychnine proved to be unsatisfactory for testing the comparative anticonvulsant action of barbiturates. Picrotoxin was a satisfactory agent for measuring this action. Ten male rats of the strain used weighing from 66 to 80 grams inclusive proved to be a sufficient number to give consistent results. The barbiturates with short duration of anticonvulsive activity were studied with a standard interval determined at twenty minutes after the administration of the barbiturate. Those with medium and long duration of anticonvulsive activity were studied with a standard determined at one hour. Loss of anticonvulsive activity was usually continuous and varied from an hour to four days for complete loss. With two compounds studied there was an early loss of activity followed by a partial regaining of anticonvulsive activity before the final loss of activity. The information obtained did not replace information obtained by other methods. It is believed that this method might give supplemental information which would be useful in some instances.

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AN IN VITRO METHOD FOR DETERMINING THE RESISTANCE OF BETA HEMOLYTIC STREPTOCOCCI TO SULFADIAZINE*

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THE appearance of a number of strains of Group A beta hemolytic streptococci which are highly resistant to sulfadiazine makes their recognition by in vitro tests highly desirable. Certain in vitro procedures used heretofore for this purpose have been somewhat cumbersome for routine clinical use. We have been able to differentiate between common strains of group A streptococci which are susceptible to sulfadiazine and certain highly resistant epidemic strains by a relatively simple method. The concentration of sodium sulfadiazine required to cause a 50 per cent slowing of the growth rate of the organisms is measured; a casein hydrolyzate, beef infusion medium enriched with small amounts of serum is used.

Similar methods have been used extensively in antibiotic studies. Kohn and Harris[1] have shown that such a method is well adapted for determining the sulfonamide resistance of bacteria in media containing sulfonamide antagonists. They have carried out exhaustive studies of growth rates of bacteria in media containing sulfonamides and various sulfonamide antagonists[1-3] and have shown that in such a medium the influence of sulfonamides on bacterial growth rates is constant only if an appropriate degree of growth inhibition is measured.[1, 4] The measurement of a degree of inhibition below a certain critical level minimizes the influence both of the para-aminobenzoic acid[3] type of antagonist and of secondary types.[1]

The rationale for methods of the type proposed is discussed by Kohn[4] in a consideration of the principles involved in designing a method for study of sulfonamide antagonists and synergists. He states that the type of method advocated can be adapted for determining sulfonamide resistance of bacteria in vitro. The desirability of using a growth rate method to compensate for cultural differences of different strains of bacteria is stressed. The fallacy of reading all tests at a definite time is pointed out. The leeway afforded in the size of the inoculum and other advantages of this type of test are discussed.

PROCEDURE

Preparation of Medium.—Fat and papillary muscle are removed from fresh beef heart. The heart is ground and infused overnight in a refrigerator in 1,000 c.c. distilled water for each pound of ground heart. It is then heated and a temperature of 85° C. is maintained for thirty minutes. The fat is skimmed from the surface and the suspension is filtered through gauze and coarse filter paper. The filtrate is made up to volume with distilled water.

*Army Air Force Rheumatic Fever Control Program.
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To each 800 c.c. of the infusion, 200 c.c. vitamin-free casein hydrolyzate* in 10 per cent solution are added. The mixture is warmed to room temperature or above, and to each 1,000 c.c. the following chemicals are added:

Sodium chloride	2.0 Gm.
Dextrose	2.0 Gm.
Na_2HPO_4 12 H_2O	1.0 Gm.

The pH is adjusted to 7.6 and the material brought to boiling. It is then filtered and the pH is again checked.

From 16 to 18 c.c. are distributed in Nessler type pyrex tubes and autoclaved at 121° C. for fifteen minutes. The final pH will be from 7.3 to 7.4. Values below this and above 7.8 are unsatisfactory.

The medium may be stored in the refrigerator until it is needed, at which time 1 per cent sterile rabbit, human, or horse serum is added. The serum need not be inactivated in the water bath.

Preparation of Sulfadiazine Tubes.—A solution of sodium sulfadiazine in N/100 NaOH, which contains 1,280 mg. sulfadiazine per 100 c.c., is prepared. By appropriate dilutions with N/100 NaOH, solutions of 10, 40, 160, 320, and 640 mg. per cent sulfadiazine are also prepared.

Each test requires seven Wassermann type test tubes. One contains 0.25 c.c. distilled water; the other six contain, respectively, 0.25 c.c. of 10, 40, 160, 320, 640, and 1,280 mg. per cent sodium sulfadiazine. These tubes are plugged and sterilized in an Arnold sterilizer on two successive days for an hour. They may be stored indefinitely if evaporation is prevented.

Equipment Necessary for Each Test.—

1. From 16 to 18 c.c. sterile casein broth.
2. Kahn type test tube containing 0.5 c.c. sterile casein broth.
3. Fifteen- to eighteen-hour culture of test organism in casein broth.
4. Control and six sulfadiazine tubes (see under Preparation of Sulfadiazine Tubes).
5. Sterile plugged graduated 10 c.c. pipette.

Procedure.—Between 8 and 10 A.M. an eighteen-hour culture of the organism is agitated several times and a loopful (platinum loop of from 2 to 3 mm. inside diameter) is transferred to 0.5 c.c. warm casein broth. (It is desirable to keep all broth as near 37° C. as is practical.) After agitation, a loopful of material is transferred from the 0.5 c.c. tube to a tube containing 18 c.c. of warm broth.

This 18 c.c. tube of inoculated broth is mixed thoroughly either by being aspirated with bulb and pipette ten times or by being placed for five minutes in a Kahn shaker. Then 2.25 c.c. of the inoculated broth are transferred to each of the control and six sulfadiazine tubes. The final sodium sulfadiazine concentration of each tube will be 0, 1, 4, 16, 32, 64, and 128 mg. per cent.

All control tubes are placed in one rack and the sulfadiazine tubes in another rack on the same shelf of the incubator at from 36 to 37° C. It is most important that the temperature is not allowed to go above 37° C.[5]

*General Biochemicals, Inc., Chagrin Falls, Ohio.

The rack of control tubes is inspected during the evening and the time at which each becomes visibly turbid is noted. For most clinical work, it is sufficient to inspect the tubes at about 8 and 11 P.M.

Reading and Reporting Tests.—The sulfadiazine tubes are read when they have incubated for approximately twice as long as the time required for the control tube to become turbid. It is assumed that if an inoculum requires twice as long to achieve visible turbidity in a sulfonamide as in a control tube, the growth rate in the former was only half that of the control. We have adopted the following routine: If the control tube was turbid by 8:00 P.M., the corresponding sulfonamide tubes were examined at from 8:00 to 9:00 A.M. At

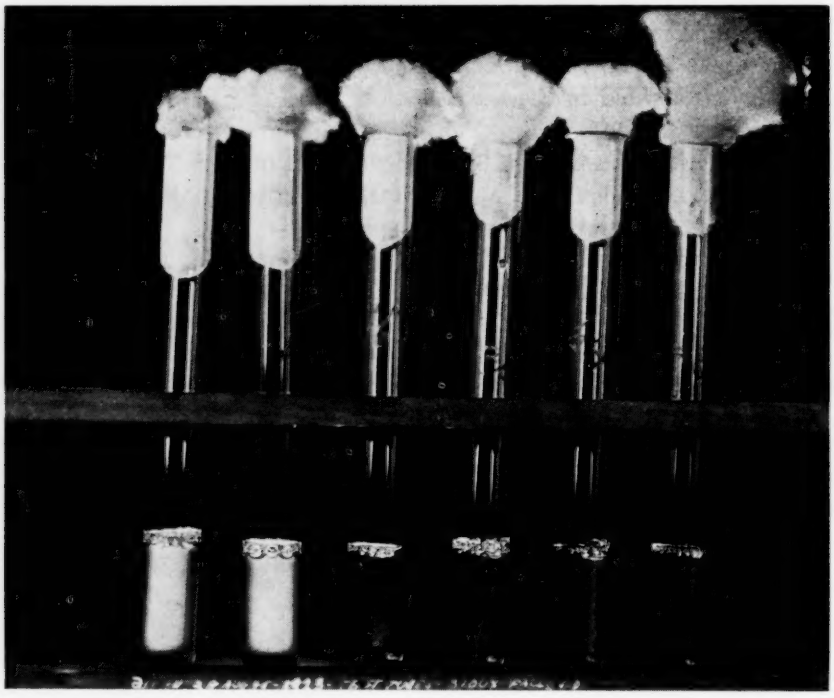

Fig. 1.—Sulfadiazine-susceptible strain of Group A beta hemolytic streptococcus showing end point at 4 mg.% sulfadiazine. Many strains will show no visible growth in any sulfonamide tube. (Official photograph, United States Army Air Forces.)

this time all strains which had grown out in media containing as much as 128 mg. per cent sulfadiazine were tentatively reported "resistant," pending culture for purity of the highest sulfonamide tube showing growth. Sulfonamide tubes for which the control tube became turbid by 11:00 P.M. were read at noon. The few sulfonamide tubes for which the corresponding control became turbid after 11:00 P.M. and before the following morning were arbitrarily read late the following afternoon.

Before the six sulfonamide tubes are examined, they should be shaken vigorously for a few seconds. It is usually noted that all tubes below a certain sulfadiazine concentration will show a heavy turbidity, while those above that level will be clear (Fig. 1).

The highest sulfonamide-containing tube showing definite turbidity is regarded as the concentration of sodium sulfadiazine causing 50 per cent inhibition of the growth rate and is so reported. Tubes in which the clump of organisms is so small as not to be obvious after shaking are regarded as clear. Rarely the end point is not definite, but an error of one tube does not usually influence the interpretation of results.

Known sulfadiazine-susceptible and -resistant strains are tested routinely (Figs. 1 and 2).

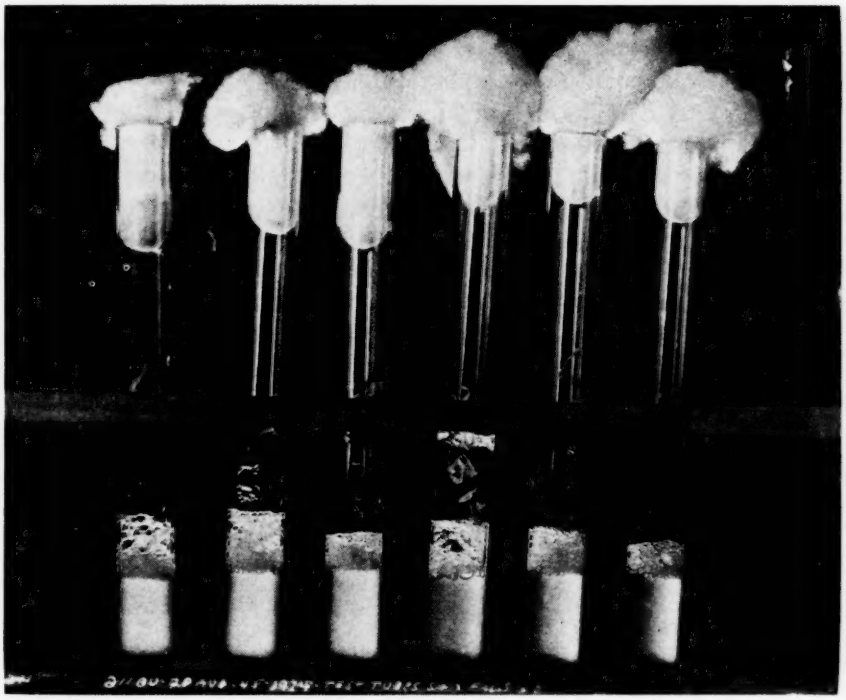

Fig. 2.—Sulfadiazine-resistant strain of Group A type 17 beta hemolytic streptococcus showing growth in all sulfadiazine tubes from 1 to 128 mg. per cent sulfadiazine. (Official photograph, United States Army Air Forces.)

RESULTS OBTAINED

Group A streptococci from the Denver area and a number of Air Force installations throughout the country were tested at Buckley Field, Colorado, by the proposed method. The clinical response to sulfadiazine of a number of the strains tested was known. An epidemic of streptococcal infections caused by a highly sulfadiazine-resistant strain of type 17 streptococcus (reported elsewhere) furnished a large number of clinical cases with which to check in vitro results. This limited experience indicated that organisms would be susceptible to therapeutic administration of sulfadiazine if they grew only in 4 mg. per cent concentration of sulfadiazine or less and resistant to therapy if they were able to grow in concentrations of 64 mg. per cent or more. Intermediate values for in vitro resistance gave less satisfactory clinical correlation. The parallel between clinical response and in vitro resistance with most strains of Group A

streptococci was close because most strains encountered by us were either markedly sulfadiazine-susceptible or -resistant.

Certain strains of streptococci of varying degrees of sulfadiazine resistance were tested from thirty to fifty times in different batches of medium. Variation in results of more than one tube occurred infrequently. The test is therefore sufficiently accurate to be of clinical value.

The Wilson[6] method of determining sulfadiazine resistance of streptococci in vitro has been used by Shott[7] at another laboratory. A series of 318 Group A streptococci of different types were tested by the proposed method and the Wilson method. Radical differences between the two methods prevent direct comparison of sulfonamide values obtained. There was agreement on the in vitro resistance to sulfadiazine for 94 per cent of the strains tested. It is of interest that both laboratories working independently were in agreement that streptococci of types 3, 17, 18, 19, and 30 are sometimes sulfadiazine resistant in vitro. Shott[7] also reported that strains of types 1 and 14 were resistant in vitro, these types having been encountered infrequently by us. No Group A streptococci other than strains of the five types mentioned were found resistant by the proposed method.

SUMMARY

1. A simple method of determining the sulfadiazine resistance of Group A beta hemolytic streptococci is described.

2. The test is based on the observation of Kohn and Harris, that sulfonamide antagonists do not influence results greatly if an appropriate degree of growth inhibition is measured.

The author is greatly indebted to Pfc. H. I. Kohn for instructions in designing this test.

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EFFECT OF METALS ON GIEMSA STAIN SOLUTIONS IN FIFTY PER CENT GLYCEROL AND METHANOL MIXTURE

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DURING World War II, one batch of Giemsa stain solution prepared for the use of the Naval Medical Corps deteriorated in service at various stations. Officers complained that all eosin staining was lost. This lot had given satisfactory staining and spectroscopic tests before issuance, and retained samples in the Naval Medical School and in the National Institute of Health still gave satisfactory tests.

Examination of spectroscopic data revealed a marked proportionate decrease in optical density at the eosin peak of from 516 to 518 mμ in the case of the two samples giving unsatisfactory stains. Heating the solutions and shaking them frequently for several days failed to remedy this defect. It was thought that exposure to outdoor winter temperatures might have occasioned precipitation of eosinates, thus decreasing the relative quantity of eosin remaining in solution, since Giemsa stain normally contains an added excess of thiazine dyes over the neutral eosinates.

At this point in the investigation, O. C. Western, Lt., and E. E. Ozburn, Lt. (j.g.), both M.C., U.S.N.R., in the chemistry laboratory of the Naval Medical Center, observed a yellowish incrustation on the tin-foil seals lining the screw caps of the Giemsa stain bottles and found that when this material was washed off in water, a yellowish pink solution was obtained.

Following these observations, we obtained some of the incrusted tin-foil cap liners and extracted them with aqueous sodium carbonate solution. These yielded red solutions with a yellow fluorescence on dilution. Spectroscopically, after dilution with water, they gave maximum absorption at λ 516; the 90 per cent density band was 11 and 12 mμ wide and medians were found to be 515.2 and 516.3. These data lie within the range given by usual samples of eosin Y in distilled water.

Next, some uncorroded tin-foil cap liners were immersed in laboratory samples of Giemsa stain at 37° C. When a 1:500 dilution in distilled water was examined spectroscopically, this stock stain gave a ratio of eosin density to thiazine density (DE/θ) of 0.571. In sixteen hours, this value reached 0.486; in seven days, 0.199. Since, in accordance with the Beer-Lambert law, optical density is directly proportional to the concentration of the solution, this fall in density at the eosin peak, in relation to the density at the thiazine peak, indicates a considerable loss of eosin from the stock solution. This experimentally altered sample presented the same loss of eosin staining as was observed in the deteriorated samples returned for re-examination.

From the Pathology Laboratory, National Institute of Health.
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*Medical Director, U. S. Public Health Service.

TABLE I. SPECTROSCOPIC DATA ON VARIOUS SAMPLES OF ONE LOT OF GIEMSA STAIN

DESCRIPTION OF SAMPLES	DATE	EOSIN			THIAZINE COMPONENT			
		λ	D	R	λ	D	W	M
Original pre-issue examination	8/31/44	518	.264	.574	660	.460	21.5	656.6
Re-examination of above sample	2/20/45	516	.170	.555	660	.305	25.4	654.8
Same, heated	2/27/45	516	.202	.525	655	.385	24.9	653.4
Returned unsatisfactory sample	2/20/45	516	.100	.372	657.5	.270	25.4	654.5
Same, heated	2/27/45	516	.125	.352	655	.355	24.7	653.4
First sample from Naval Medical School Stores	2/20/45	518	.082	.246	657.5	.332	23.3	655.1
Same, heated	2/27/45	516	.090	.262	655	.345	24.9	653.8
Second sample from Naval Medical School Stores	2/20/45	518	.214	.560	657.5	.382	25.4	654.8
Same, heated	2/27/45	516	.221	.525	655	.421	23.2	654.3

λ = Absorption maximum.
D = Optical density at absorption maximum.
R = D at eosin absorption maximum divided by D at thiazine absorption maximum.
W = Width of absorption band in which D exceeds 90 per cent of its maximum.
M = Median of absorption band in which D exceeds 90 per cent of its maximum.

The composition of the tin-foil* liners employed was unknown to us at that time. Therefore, we placed small quantities of various pure metals in 10 c.c. quantities of 0.8 per cent Giemsa stain in equal volumes of glycerol and methanol and incubated these at 37° C. In addition controls were used with no added material and with cardboard, cork, and rubber.

In the five controls, the ratio DE/Dθ remained above .500 except for the forty-four day cardboard sample; it became .415. Similar behavior was noted with cobalt, chromium, lead, copper, silver, and one sample of brass. With calcium and magnesium there was a pronounced shift of the thiazine absorption band from the initial 658.2 median to 605.9 and 620.9 for the first two, respectively; with two samples of cadmium, the shift was to 638.5 and 616.5. This is interpreted as the usual effect of alkali.† With calcium, the DE/Dθ ratio rose to 1.32 and 1.062 at fourteen and twenty-eight days, respectively; with magnesium, it remained in the usual zone; while with cadmium it fell in twenty-eight days to .323 for the first and to .290 for the second sample. With the second sample of brass, two samples each of aluminum, zinc, tin, arsenic, and antimony, the DE/Dθ reached values below 0.300 in from fourteen to twenty-eight days or longer; with aluminum, sixty-three days were required and with arsenic, seventy-seven days (one sample each). The lowest values for the DE/Dθ ratio were reached with zinc (.162, .129) and aluminum (.186, .172).

The two most active metals in the electrolytic series produced primarily alkali effects, probably because of their rapid conversion to hydroxides, and there was little or no adsorption of eosin. The lower metals in the electrolytic series apparently did not decompose the eosinate, while the middle members actively decomposed the eosinates and removed the eosin from solution.

With the exception of the calcium, cadmium, and magnesium samples noted, the thiazine absorption band median either remained fairly stationary between 658 and 650 or shifted moderately to the left (into the 640's); with one sample

*The composition of the tin foil used for these cap liners was furnished us from the manufacturer: tin 99.7 per cent; lead, not over 0.12 per cent; copper, 0.03 per cent; arsenic, 0.103 per cent; antimony, 0.08 per cent; and iron, not over 0.04 per cent.

†Lillie, R. D.: The Deterioration of Romanowsky Stain Solutions in Various Organic Solvents, Pub. Health Rep., No. 178 (Suppl.), 1944.

TABLE II. EFFECT OF INCUBATION OF GIEMSA STAIN AT 37° C. WITH VARIOUS METALS ON ABSORPTION SPECTRUM

	DAYS TIME	λ E	λ Θ	90% DΘ		DE/DΘ
				W	M	
Control	0	516	660	27.9	658.2	0.655
	14	516	652.5	24.9	652.2	.769
	28	516	652.5	30.1	647.6	.765
Second Control	14	516	657.5	25.6	652.5	.711
	28	516	652.5	26.4	650.7	.547
Cardboard Control	14	518	652.5	19.3	650.8	.565
	44	517	650	28.6	647.8	.415
Cork Control	14	516	652.5	31.7	649.6	.555
	44	517	650	28.8	648.1	.619
Rubber Control	14	516	657.5	28.2	649.6	.619
	44	516	649	31.0	646.0	.730
Tin						
First sample	14	520	657.5	26.0	656.2	.249
Second sample	14	516	655	26.9	652.4	.270
	28	516	657.5	21.1	656.5	.250
Cadmium						
First sample	14	516	652.5	36.3	643.6	.475
Second sample	28	518	645	35.1	638.4	.323
	77	518	640	30.6	636.5	.243
	14	517	632.5	39.3	631.5	.320
	28	524	617.5	35.4	616.5	.290
Zinc						
First sample	13	518	660	26.5	661.0	.162
Second sample	14	504	655	23.6	654.1	.151
	28	498	659	20.6	657.4	.129
Aluminum						
First sample	13	521	660	31.6	652.4	.482
Second sample	63	516	645	33.4	640.0	.186
	14	516	651	26.4	648.5	.274
	28	519	650	20.0	650.0	.172
Magnesium	14	516	640	40.4	632.5	.859
	28	515	622.5	33.9	620.9	.683
Calcium	14	516	610	31.5	607.3	1.320
	28	514	605	30.1	605.9	1.062
Iron	14	520	657.5	26.5	655.0	.547
	63	518	650	31.0	646.2	.387
Cobalt	14	516	652.5	32.3	648.2	.779
	28	517	647.5	31.9	643.5	.699
Chromium	14	516	657.5	25.6	653.6	.730
	28	517	652.5	19.9	652.7	.677
Lead	14	520	660	26.9	657.4	.705
	63	516	650	35.5	643.1	.746
Copper	14	518	660	28.2	656.2	.673
	63	514	652.5	32.8	647.5	.614
Silver	14	517	657.5	25.2	655.3	.750
	28	514	657.5	23.4	654.6	.520
Brass						
First sample	14	516	660	27.2	654.1	.750
Second sample	28	516	650	25.9	650.4	.539
	14	515	646	28.8	645.0	.384
	28	516	649	29.3	646.0	.200
Arsenic	14	516	655	27.5	653.8	.545
First sample	28	516	655	24.6	652.6	.338
Second sample	77	516	647.5	27.7	649.0	.209
	14	517	650	32.5	643.4	.625
	28	522	645	34.4	634.5	.206
Antimony	14	516	662.5	24.6	654.3	.296
First sample	28	514	656	25.6	658.1	.228
Second sample	77	516	656	24.7	654.0	.299
	14	516	657.5	24.7	652.8	.246
	28	512	660	23.0	654.5	.209

λ = Absorption maximum.
E = Eosin.
Θ = Thiazine.
D = Optical density at absorption maximum.
W = Width of absorption band in which Density (D) is over 90 per cent of its maximum.
M = Median of absorption band in which Density (D) is over 90 per cent of its maximum.

with arsenic, to 634.5. With zinc, however, the absorption band median shifted to 661 with one sample at thirteen days, and from 654 at fourteen days to 657.4 at twenty-eight days with the other.

In further exploration of this atypical shift, samples of thionin eosinate and of azure A eosinate in 1 per cent solutions in glycerol methanol 50:50 volume mixture were incubated at 37° C. with metallic zinc. The shift of the ratio DE/Dθ occurred as before, going from 1.000 to 0.399 in twenty-eight days with thionin eosinate and from .593 to .215 in two months with the azure eosinate. The thionin absorption band median remained constant in position between 595 and 599, both with and without zinc. The azure A absorption band median shifted from 622.9 to 638.0 in two months with zinc but remained between 621 and 623 without it.

The exact explanation of this phenomenon remains obscure. Formation of ethyl thionins has been suggested.

CONCLUSION

Various metals have the property of decomposing thiazine eosinates in glycerol methanol solution and removing the eosin from those solutions. These are relatively active metals in the electrolytic series. However, alkali earth metals appear to act purely as alkalies.

Zinc acts on methyl thionins in glycerol methanol solution, but not on thionin, to shift their spectral absorption bands toward the red end of the visual spectrum.

The use of tin foil or other foils containing tin, zinc, or cadmium for lining screw caps for Giemsa stain is contraindicated. Copper foils containing appreciable admixtures of zinc or tin seem to be similarly contraindicated. Paraffined cardboard seems to be the most innocuous and generally satisfactory lining material for such caps.

Acknowledgment is due to D. Plotka, for reading the absorption spectra and doing part of the mathematical calculation of derived spectroscopic data as well as for other technical assistance.

BOOK REVIEWS

Hematology for Students and Practitioners. By *Willis M. Fowler*, A.B., M.D., Professor of Internal Medicine, University of Iowa, Iowa City, Iowa; with a chapter by *Elmer L. DeGowin*, A.B., M.D., Assistant Professor of Internal Medicine, University of Iowa, Iowa City, Iowa. Paul B. Hoeber, Inc., New York, 1945. Price $8.00. Cloth with 499 pages.

In this new volume on clinical hematology, Dr. Fowler presents, from the standpoint of the internist, the hematological viewpoint which he has exemplified in his own practice, teaching, and research at the University of Iowa in recent years. "Because of the wide variety of lesions which produce alterations in the circulating blood, hematology cannot be too definitely separated from the parent field of internal medicine, and diagnosis of diseases of the blood should be *based on a consideration of the patient as a whole, not on examination of the blood alone.*" (Reviewer's italics.)

To the undergraduate medical student and the modern-day, intelligent practitioner of medicine, the current "theories" underlying the etiology and therapy of human disease are usually considered to be less essential than to the active investigator. On this assumption, sufficient background, devoid of detailed discussions, and adequate illustrative plates, both colored and black and white, with pertinent tables and graphs are provided to support the text and to illuminate the practical instructions given and conclusions drawn. With the same objectives in mind, the bibliographic references are brief and to the point, are seldom interjected into the text, and reflect the critical selection and judgment of the author.

The discussion of the anemias is excellent, including a timely chapter on the anemia secondary to blood donations, which provides the backdrop for Elmer DeGowin's thorough and authoritative discussion of all that has been learned in World War II about blood transfusion and the therapeutic use of blood derivatives. The present reviewer has yet to see anywhere so succinct and complete a résumé of this most important subject, which is of utmost significance to *every* physician-surgeon, internist, and medical specialist alike, of whatever particular interest. The basis for donor selection, the technics of collection, preservation, and typing of blood, including the Rh factor, the organization of a blood bank, the indications and contraindications for the use of blood and/or blood derivatives and substitutes, and posttransfusion reactions, their cause, prevention, and treatment, are all treated from the most practical clinical standpoint.

The differential diagnostic approach to the hemorrhagic and purpuric syndromes is clearly presented, and a very real service is performed in bringing some order out of a considerable degree of chaos in the thinking of many medical students and practicing physicians in these areas.

In contrast to the primary blood dyscrasias appears a brief chapter on the blood picture in a miscellaneous group of infections, which will be helpful to the general clinician.

An innovation worthy of emulation and elaboration is the separate chapter in this work devoted to a special consideration of the blood picture in infancy and childhood. Diagnostic and prognostic interpretations are decidedly conditioned by the age of the patient, and a recognition of this will avoid many errors in clinical judgment and therapeutic advice.

The volume is closed with a short chapter on routine laboratory procedures and an index facilitating rapid reference to specific detailed information. This volume reflects the improvement in format and quality of paper stock which the postwar period will now make possible.

Dr. Fowler has admirably accomplished his stated objectives.

CHARLES A. DOAN.

Pulmonary Edema and Inflammation. Harvard University Monograph in Medicine and Public Health No. 7. By *Cecil K. Drinker*, M.D., D.Sc., Professor of Physiology, School of Public Health, Harvard University, Boston, Mass. Harvard University Press, Cambridge, Mass. Price $2.50. Cloth with 106 pages.

This small volume constitutes a series of four lectures delivered at the Bowman Gray School of Medicine in December, 1944, together with a fifth chapter on artificial respiration. Although the title of the book is perhaps somewhat misleading, since only the briefest mention is made of inflammation, the text is packed with valuable information and is written in a pleasant informal style punctuated by frequent touches of delightful humor.

The first lecture, dealing with structure, describes the vast capillary network of the lung and defines its relation to the more than 750 billion alveoli. The structure and function of the pulmonary lymphatics are authoritatively discussed and there is included a detailed description of the author's own method of cannulating the right lymphatic duct of the dog. Since in most dogs practically all of the lymphatics from both lungs drain into the right duct, it is possible to study pulmonary lymphatic flow by means of cannulating this delicate vessel.

In discussing the histology of the lung, Drinker quotes widely from the writings of William Snow Miller whose volume on the anatomy of the lung has for many years been accepted as the most authoritative treatise on the subject. It is of interest, therefore, that the author accepts without reservation the evidence presented by Loosli concerning the existence of the interalveolar communications known as the pores of Cohn. Miller, who contended that each alveolus was lined with a very thin layer of flat epithelium, claimed that these minute communications between the alveoli could be found only in pathological lung tissue and were absent in the normal lung. To refute this earlier claim, Drinker quotes extensively from Loosli's experiments and even goes so far as to publish two of his camera lucida drawings illustrating the pores of Cohn. The acceptance of this evidence is of particular significance since Drinker fails to mention the equally conclusive experiments of the same author disproving Miller's contention that each alveolus is lined with epithelium. Loosli has clearly demonstrated that the alveolar walls are made up only of capillaries supported in a network of reticulum and he has pointed out that the pores of Cohn merely represent the holes which normally exist in this network. To the reviewer at least, it appears illogical to accept the existence of the pores of Cohn and at the same time contend that the alveoli are lined with epithelium. Drinker not only ignores the important observations of Loosli regarding the structure of the alveolar walls, but he also makes frequent reference to the epithelial lining of the alveoli in interpreting the consequences of pulmonary edema.

The second lecture discusses physiological factors relating to pulmonary edema. At the outset, the author clearly states his belief that: "Simple pulmonary edema and the more serious pulmonary exudation depend more upon alterations in the permeability of the lung capillaries than upon complicated pressure relations in the pulmonary circulation." He presents considerable experimental evidence to support this view and emphasizes the role of anoxia in causing increased capillary permeability. Although the author's reasoning appears to be entirely sound, most of the evidence presented is indirect, and the reader is left with the impression that more direct methods of experimentation must be employed before the statement can be conclusively proved. Of greatest interest in this lecture is the discussion of an unnamed derivative of thiourea which apparently exerts a selective action upon the pulmonary capillaries, causing little, if any, change in the other capillaries of the body. The drug, which is extremely insoluble in water, when injected intravenously, causes profound pulmonary edema. The fluid leaking from the pulmonary capillaries is at first carried off by the lymphatic drainage, but eventually the leakage becomes so marked that the lymphatics can no longer handle the load and pulmonary edema ensues. This compound should in the future be of great value to physiologists in elucidating the exact mechanism of pulmonary edema.

In the third lecture Drinker discusses the effect of breathing movements upon lymphatic flow in pulmonary tissue. Cleverly designed experiments are described which indicate that increased inspiratory and expiratory efforts augment the flow of lymph from the lung. The importance of position as the cause of blood stasis and atelectasis is emphasized. The author warns against the dangers of allowing stasis to develop in hospitalized patients and mentions some of the more practical methods of avoiding this usually unnecessary complication.

The fourth and final lecture deals with a discussion of "Preventive and Therapeutic Measures in Asphyxiating Pulmonary Disease." This lecture opens with two delightful paragraphs, the first of which deals with the relationship between liberalism and the scientific mind and the second which contains the following description of the characteristic Harvard Professor: "He wears a red necktie; his trousers do not fit; and he is invariably right!" There follows a scholarly but thoroughly practical discussion of the use of oxygen and carbon dioxide in medical therapy. A warning is sounded against the indiscriminate use of morphine and brief comments are made concerning the use of helium and positive pressure oxygen. The physician is urged to employ oxygen early and to educate his patients and their families toward the point of view "that the words oxygen and undertaker are not synonymous."

The final chapter on artificial respiration is a masterpiece and should be read by all medical students and house officers. It not only discusses in some detail the various practical methods of administering artificial respiration, but it also sets forth, in the clearest terms possible, the physiological principles involved in emergency resuscitation. The author points out that mechanical pulmotors are in the main dangerous and ineffective and he emphasizes that immediate manual artificial respiration or mouth to mouth breathing should be employed in respiratory emergencies.

This volume constitutes an important addition to medical literature and maintains the high standards set by previous Harvard University Monographs in Medicine and Public Health.

W. B. Wood, Jr.

The Hair and Scalp. A Clinical Study (With a Chapter on Hirsuties). By *Agnes Savill*, M.A., M.D. (Glasg.) F.R.C.P.I. Third edition. Williams & Wilkins Company, Baltimore, Md., 1945. Price $4.75. Cloth with 304 pages and 54 figures.

Agnes Savill has revised and enlarged the new edition of her excellent monograph on the hair and scalp. The opening chapter on the structure and physiology of the hair is a complete presentation of our basic knowledge of this subject. Two excellent chapters follow on canities and the care of the hair. One can challenge, however, the implication that dandruff is infectious.

Permanent waving, singeing, bleaching, and dyeing of the hair are described from the scientific standpoint.

There is an excellent and informative chapter by W. T. Astbury on the molecular structure and elastic properties of hair. It should be read and studied by all physicians who are interested in the hair.

Dr. Savill's descriptions of the many diseases of the hair and scalp are interesting and informative. The relationship of internal diseases and scalp and hair affections are emphasized.

New additions to the present volume are sections on cleansing agents, pigmentation, recent vitamin and endocrine discoveries, congenital defects, rare tumors of the scalp, etc.

Hamilton Montgomery (Mayo Clinic) has evidently inspired additions and changes in the present edition. More credit has been given to American investigators than in previous editions. This will no doubt be further improved in subsequent editions as the volume still lacks reference to some important American contributions.

This book can be highly recommended to all dermatologists and physicians who are at all interested in diseases of the hair and scalp.

Richard S. Weiss.

Virus as Organism (Harvard University Monograph in Medicine and Public Health No. 8). By *Frank M. Burnet*, M.D., F.R.S., Director, Walter and Elize Hall Institute of Research in Pathology and Medicine, Melbourne, Australia. Harvard University Press, Cambridge, Mass. Price $2.00. Cloth with 134 pages.

In this volume is set down the substance of the Edward K. Dunham Lectures delivered by the author at Harvard University in 1944. In that series of lectures, Dr. Burnet set himself the task of considering the viruses not as macro-molecules or autocatalytic enzymes, or parasites, but as biological entities occupying a place in the cosmos of living things. He explains the basis for his faith that viruses are microorganisms which have evolved by parasitic degeneration from larger microorganisms, many of them in all probability from bacteria. It is his thesis that they still show evidence in their chemical structure of conformity with the general pattern of living material and that their behavior must be interpreted mainly in terms of biological concepts. He examines the general problem of evolution and change in virus disease and the reaction of the host to virus infection.

Spontaneous generation of viruses is dismissed as a concept without supporting evidence and without necessity for a consistent philosophy. The mutation of viruses is discussed and the biological requirements for survival of a mutant strain are listed. It is pointed out that excessive virulence may be just as much of a handicap in the struggle for survival of a virus as inadequate virulence. A perfect parasite is one which does not harm its host too much, multiplies at a rate sufficient to insure its survival, and is able to pass from host to host without excessive difficulty. The special problems of a virus in this respect are pointed out. The author shows that from a biological point of view "virus" must be considered the total mass of virus in existence, not the small collection of particles in a single cell or even a single animal. Herpes simplex, poliomyelitis, psittacosis, the smallpox group, yellow fever, and influenza serve as convenient and important examples of virus disease to which biological concepts are applied.

Dr. Burnet is not only an acknowledged leader in virus research, but also a serious thinker about the larger aspects of disease and a forceful and entertaining writer. This volume will have little interest for the person interested only in the chemistry or physics of viruses, but it is highly recommended to the thoughtful physician as well as the biologist interested in the interrelation of organisms.

ROBERT F. PARKER.

Organic Preparations. By *Conrad Weygand*, Professor at the University of Leipzig. Interscience Publishers, Inc., New York. Price $6.00. Cloth with 534 pages.